ISSN 0377-9335

entomon

A Quarterly Journal of Entomological Research

Vol. 9 JUNE 1984 No. 2

PUBLISHED BY
THE ASSOCIATION FOR ADVANCEMENT OF ENTOMOLOGY
DEPARTMENT OF ZOOLOGY, UNIVERSITY OF KERALA, KARIAVATTOM
TRIVANDRUM, INDIA 695 581

ENTOMON

Entomon is a quarterly journal of the Association for Advancement of Entomology issued in March, June, September and December, devoted to publication of research work on various aspects of insects and other land arthropods.

Address MS and all editorial correspondence to the Managing Editor, *Entomon*, Department of Zoology, University of Kerala, Kariavattom, Trivandrum, India 695 581.

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entomon

Volume 9 June 1984 Number 2

CONTENTS

Entomon, 1984, *Vol.* 10, *No.* 2, *pp.* 81 *to* 86

BLOOD MEAL SIZE OF *ANOPHELES STEPHENSI* LISTON AND *ANOPHELES CULICIFACIES* GILES DURING SUCCESSIVE GONOTROPHIC CYCLES

V. K. BHASIN, S. CHITRA AND M. K. K. PILLAI*

Department of Zoology, University of Delhi, Delhi India - 11007,

(Received 26 December 1983)

Haemiglobincyanide method employed to determine replete blood meal size indicates that *Anopheles stephensi* took significantly more blood compared to *Anopheles culicifacies*. Amount of first replete blood meal per 4-day-old mated female in *A. stephensi* is 4.8 mg and only 1.3 mg in *A. culicifacies*. No significant difference in first replete blood meal was observed between mated and unmated females or mated females of different ages. However, second and third replete blood meals were significantly larger than the first. Females laid significantly more eggs In first gonotrophic cycle compared to the second or third. There was a significant increase in dry body weight of females during successive gonotrophic cycles. Implication of larger second and third blood meal amounts were discussed.

(Key words: blood meal size, *Anopheles stephensi*, *A. culicifacies*, gonotrophic cycles)

INTRODUCTION

Blood meal size in female mosquitoes to a considerable extent, determines acquisition and transmission of parasites (HOVANITZ, 1947; JEFFERY, 1956), refeeding behaviour (EDMAN *et al.*, 1975; KLOWDEN & LEA, 1978) and biotic potential (ROY 1936; WOKE *et al.*, 1956; EDMAN *et al.*, 1975). The amount of blood ingested thus affects the physiology of the female mosquito as well as its vector potential in natural population. It is likely that the amount of blood taken varies in different species of mosquitoes. However, little is known about the size of the replete blood meal in different mosquito species. *Anopheles stephensi* and *A. culicifacies* are incriminated vectors of malaria in India (KRISHNAN, 1961). Therefore, it was considered of interest to evaluate in the laboratory the replete blood meal intake of these two species during successive gonotrophic cycles and to assess the relationship between the blood meal size and fecundity and body weight of female mosquitoes.

* Correspondence: Dr. M. K. K. Pillai, Professor, Department of Zoology, Delhi University, Delhi - 110 007.

MATERIALS AND METHODS

Strains of *A. stephensi* and *A. culicifacies* employed in the present investigations were raised from engorged adult females collected from cattle sheds in Okhla village in Delhi during April-May, 1979 and Mandora village in Haryana state, India, during May 1980, respectively. Mosquitoes were maintained in the laboratory at $28 \pm 2°C$ and $80 \pm 5\%$ RH under 12 hr light and 10 hr dark photoperiod with 90 min each of dawn and dusk to simulate out-door field condition. Adult mosquitoes were held in muslin cages ($30 \times 30 \times 30$ cm) on water soaked split raisins. Females were blood fed on albino rats. Filter paper lined bowls containing water were kept inside the cages for oviposition. Larvae were reared in dechlorinated water on a diet

containing a mixture of finely ground dog biscuits and yeast (3:2).

Females for blood meal size determination were deprived of raisins for 12 hr and anal opening of *A. stephensi* blocked with melted paraffin wax before feeding on shaved, restrained laboratory rats. Immediately after feeding, females were carefully removed from the cage by means of an aspirator and stored frozen in a deep freeze (-20°C) for subsequent use in blood meal size determinations. First replete blood meal size of mated females (2-, 4-, 8-day-old) and unmated females of 4-day-old of *A. stepensi* and 4-day-old mated females of *A. culicifacies* were determined. For measuring second and third replete blood meal size, mated females after first and second oviposition respectively were used.

The haemiglobincyanide (HiCN) method of BRIEGEL *et al.* (1979) was used to measure the blood meal size of the mosquitoes. Fully blood-fed, stored frozen females were dissected and the blood filled midgut was placed in a homogenizer containing 1 ml of Drabkins reagent (BRIEGEL *et al.*, 1979). Care was taken to remove the malpighian tubules. The midgut with blood was thoroughly ground with a pestle to assure complete elution of haemoglobin. Samples were incubated at room temperature for 20 min. A 200 μl aliquot was transferred to a microcuvette and percentage transmission was read at 540 nm using a Spectronic-20 colorimeter. A standard curve of host blood weight *vs* optical density was prepared for each experiment and was used to estimate the weight of the blood meal in each instance. In each determination fifteen or more of females were used (Table 1). Statistical significance between various groups was determined by Student's *t* test.

TABLE 1. Blood meal size of *A. stephensi* and *A. culicifacies*.

S. No.	Status of females	Replete blood meal	Blood meal size in mg/female Mean ± S E*
	A. stephensi		
1.	2-day-old mated	first	4.73 ± 0.21 (26)
2.	4-day-old mated	first	4.82 ± 0.15 (29)
3.	4-day-old unmated	first	3.97 ± 0.25 (25)
4.	8-day-old mated	first	4.93 ± 0.14 (17)
5.	After first oviposition, mated	second	6.08 ± 0.14 (25)
6.	After second oviposition, mated	third	6.00 ± 0.21 (30)
	A. culicifacies		
7.	4-day-old mated	first	1.37 ± 0.10 (18)
8.	After first oviposition, mated	second	2.64 ± 0.09 (29)
9.	After second oviposition, mated	third	2.80 ± 0.13 (17)

*The figures in the parentheses indicate the number of females used in each experiment.

P values between S. No.

1 and 2 P 0.5	7 and 8 P 0.005
1 and 4 P 0.5	7 and 9 P 0.005
2 and 3 P 0.1	8 and 9 P 0.5
1 and 5 P 0.01	2 and 7 P 0.001
1 and 6 P 0.025	5 and 8 P 0.001
5 and 6 P 0.1	6 and 9 P 0.001

The number of eggs laid per female during each successive gonotrophic cycle was determined by isolating a group of mated females after their first replete blood meal. Oviposition bowls maintained for 2 days after isolation and replaced daily thereafter until no egg laying was observed for 24 hr. Surviving females were given a second blood meal and allowed to lay eggs as before. Similarly eggs laid during each gonotrophic cycle was recorded and the number of eggs laid during each gonotrophic cycle was recorded and the number of eggs laid per female was calculated. Each experiment was replicated at least thrice with fifteen or more females at start of each experiment (Table 2). The period from the beginning of one blood meal to the next was recorded as duration of one gonotrophic cycle.

Groups of ten females were used to determine dry body weight. These were kept in an oven at 100°C until a constant weight was achieved at 6 hr interval (2-4 days). The dry body weight of mated and unmated females and also of females after first, second and third oviposition was recorded. At least three replicates were used to determine the mean dry body weight.

TABLE 2. Oviposition rate of *A. stephensi* and *A. culicifacies* during successive gonotrophic cycles.

S. No.	Gonotrophic cycle	Totai No. of females used*	Total No. of eggs laid	No. of eggs per female	Duration of gonotrophic cycle in days
A. stephensi					
1.	first	95 (3)	8981	94.33 ± 3.30	7.66 ± 0.40
2.	second	69 (3)	4680	66.36 ± 4.21	6 0 ± 0.0
3.	third	64 (3)	4193	64.74 ± 6.35	5.66 ± 0.40
A. culicifacies					
4.	first	60 (4)	887	14.78 ± 1.09	
5.	second	40 (4)	483	12.07 ± 0.61	
6.	third	37 (4)	410	11.15 ± 0.60	

*The figures in parentheses indicate the number of replicates.

RESULTS AND DISCUSSION

It is clear from Table 1, that the replete blood intake of 4-day-old mated and unmated females of *A. stephensi* did not differ significantly ($p > 0.1$). Also, no significant difference was observed in the first blood meal size of 2-, 4-, and 8-day-old mated females of *A. stephensi* (P 0.5 in each case). However, the amount of blood ingested during the second blood meal was significantly more (1. 26 × greater) than the first blood meal ($P < 0.01$). Similarly, the third blood meal of *A. stephensi* was significantly larger (1.24 × greater) than the first blood meal ($P < 0.025$). However, no significant difference was observed between the replete blood meal size of the second and third gonotrophic cycles (P 0.1). Almost identical results were obtained with females of *A. culicifacies* (Table 1).

The number of eggs laid by females during first, second and third gonotrophic cycles is given in Table 2. Females laid maximum number during the first gonotrophic cycle. There was a significant reduction in fecundity in subsequent gonotrophic cycles in both the species ($P<0.001$ in *A. stephensi* and $P<0.05$ in *A. culicifacies*). Duration of the first gonotrophic cycle was observed to be the longest in *A. stephensi*.

There was a significant increase in the dry body weight of *A. stephensi* females after first oviposition compared to 4-day-old mated females deprived of blood ($P<0.05$, Table 3). Although the body weight of females increased slightly after the second and third oviposition, it was not significantly different from females after first oviposition ($P>0.1$). Also, no significant difference in body weight was found between female after second and third oviposition ($P>0.5$). or between non-blood fed mated and unmated females ($P>0.5$).

Though the gravimetic method has been used extensively to determine the blood meal size of female mosquitoes it often results in underestimation (CLEMENIS, 1963; BRIEGEL *et al.*, 1979). The haemiglobincyanide method employed in the present studies eliminates this error (BRIEGEL *et al.*, 1979). Also care was taken to seal off anal ends of *A. stephensi* to prevent the excretion of erythrocytes as it is known to excrete erythrocytes while feeding and thereafter.

Results of our study indicate that the replete blood meal volumes of *A. stephensi* is significantly more compared to *A. culicifacies*. The amount of the first replete blood meal per mated female is 4.8 mg in *A. stephensi* and only 1.3 mg in *A. culicifacies* as compared to 3.4 mg in *A. quadrimaculatus* and 2.5 mg in *A. albimanus* when determined gravimetrically (JEFFERY, 1956).

In *A. stephensi* there was no significant size difference between the first replete blood meal of mated and unmated females. Similar results were obtained in *Aedes aegypti* by KLOWDEN (1979) using the haemiglobincyanide method. Our data indicate that age does not influence the size of the first replete blood meal in *A. stephensi*. KLOWDEN & LEA (1980) found that in *Ae. aegypti*, 5-day-old females took significantly more blood than 10 or 20-day-old females.

TABLE 3. Dry body weight of females of *A. stephensi*.

Status of the females	Dry body weight of 10 females in mg. Mean ± S E
4-day-old mated and unfed on blood	3.98 ± 0.21 (10)
After first oviposition (10-day old)	5.16 ± 0.13 (9)
After second oviposition (15-day old)	6 18 ± 0.41 (3)
After third oviposition (20-day old)	6.42 ± 0.20 (3)
Unmated unfed on blood (4-day old)	3.59 ± 0.24 (6)

Figures in parentheses indicate the number of replicates

An important factor governing the capability of a mosquito is its propensity towards a second blood meal. This study clearly shows that the first replete blood meal was significantly smaller than second and third meal in both the species. A large blood meal increases the chances of acquiring parasites from an infected host (HOVENITZ, 1947; JEFFERY, 1956). *A. stephensi* and *A. culicifacies* are known to be malaria vectors par excellence in India and neighbouring countries and it may largely be due to the enhanced vector capability consequent on large blood meal size during multiple feeding.

The number of eggs laid per female was greatest during the first gonotrophic cycle even though the amount of replete blood intake was least during this cycle. But there was no correlation between the egg production and blood meal size during successive gonotrophic cycles. In each subsequent gonotrophic cycles there was a reduction in number of eggs laid per female. Similar decrease in the number of eggs during successive ovipositions was recorded in *A. quadrimaculatus* and *A. maculipennies* (LOVE, 1954; DETINOVA, 1962). Also, in *Ae. aegypii* it was estimated that each successive egg batch contained 15% fewer eggs than the one which preceded it (PUTMAN & SHANNAN, 1934).

Duration of the first gonotrophic cycle was longest. This may be due to the fact that after the first oviposition, development of the next follicles are to certain extent advanced so the time taken by these follicles to attain maturity is shortened However, in *Culex pipiens* GAABOUB & DAWOOD (1973) found an increase in the duration between second feeding and second oviposition as compared to the first.

Our data also show a significant increase in dry body weight after first oviposition compared to females before the first blood meal. This increase in dry weight of females during each gonotrophic cycle might be due to incorporation of part of the blood nutrients into the body. It is interesting to note in successive gonotrophic cycles that there was reduced fecundity, this suggests with increased age more and more blood nutrients may be incorporated into the body rather than into egg production. The increase in body weight also may be responsible for larger blood meal sizes in each successive gonotrophic cycle. BARLOW (1955) reported that heavier females of *Ae. hexodontus* ingest more blood. In the stable fly *Stomoxys calcitrans,* the larger blood meal size of females as compared to males has been attributed to the greater dry weight of female flies (SCHOWALTER & KLOWDEN, 1979).

Acknowledgements: The award of C S I R Junior Research Fellowship to one of us (S C) is duly acknowledged.

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Entomon, 1984, *Vol*, 10, *No*. 2, *pp*. 87 *to* 91

EVALUATION OF SYSTEMIC INSECTICIDES AGAINST *MYZUS PERSICAE* SULZER ON POTATO IN DECCAN PLATEAU

S. S. MISRA, B. T. RAJ[1] & M. L. TANDON[2]

Division of Entomology, Central Potato Research Institute, Shimla (H. P.), India - 171 001

(Received 26 May 1983)

Replicated field experiments were conducted at Central Potato Research Station, Rajgurunagar, Pune, for evaluating the relative efficacy of six foliar systemic insecticides viz., oxydemeton-methyl, dimethoate, thiometon, phosphamidon, monocrotophos and formothion against green peach aphid, *Myzus persicae* Sulzer, a potential vector of potato viruses. All the insecticides have been found significantly superior over water spray check in controlling the aphids. However, oxydemeton-methyl registered the control for longer duration, especially when aphid population was high. The insecticides were almost equally effective at 300 and 500 g ai/ha/spray dosages. Hence, it is suggested to use 300 g ai/ha spray dose of insecticides which will reduce the input cost and insecticidal hazards too.

(Key words: aphid, *Myzus persicae* Sulzer, oxydemeton-methyl, dimethoate, thiometon, phosphamidon, monocrotophos, formothion)

INTRODUCTION

Green peach aphid, *Myzus persicae* Sulzer is considered the most important insect vector of potato viruses so far as the cultivation of healthy seed potato is concerned (VERMA & MISRA, 1975; VERMA, 1977). This aphid transmits virus diseases like 'potato leaf roll' and potato virus 'Y' resulting in degeneration of potato stocks. In India, the losses in yield due to leaf roll virus and potato virus 'Y' are estimated to be 20 to 50 per cent and 40 to 85 per cent respectively (NAGAICH & AGRAWAL, 1969). Insecticidal control of aphid vectors has been reported as standard measure for last so many years, especially, in temperate regions of the world (BROADBENT, 1957). According to PATKAR *et al.* (1969), LAL & MISRA (1979) and MISRA *et al.* (1980), the use of modern systemic insecticides has been found effective for the control of aphids on seed crops of potato.

In Deccan Plateau regions of India, aphid free/low aphid period is very limited hence it is highly essential to eradicate or keep their population below the critical level (20 aphids/100 compound leaves) for raising healthy seed crop of potatoes. In the present investigations, efforts have been made to study the relative efficacy of some foliar systemic insecticides to evolve an effective and economical control of *M. persicae* on potato under the prevailing agro-climatic conditions of Deccan Plateau.

MATERIALS AND METHODS

Potato variety *'Kufri Chandramukhi'* was grown during *'rabi'* seasons of 1976—1977 and

[1] Entomology Section, Central Potato Research Station, Rajgurunagar, Pune.

[2] Statistics Section, Central Potato Research Institute, Shimla (H P).

1977–1978 in 4.2 m × 2.4 m plots at Central Potato Research Station, Rajgurunagar, Pune, following standard recommended agronomical practices.

Six foliar systemic insecticides viz., oxydemeton-methyl (Metasystox 25 EC), dimethoate (Rogor 30 EC), thiometon (Ekatin 25 EC), formothion (Anthio 25 EC), monocrotophos (Nuvacron 40 EC) and phosphamidon (Dimecron-100) each @ 300 and 500 g ai/ha/spray treatment, after diluting in 1000 l of water, were sprayed twice at fortnightly intervals during both the years. There were 13 treatments including control which were replicated thrice in randomized block design. First spray treatment was given soon after noticing the aphids on the crop. After 5, 10 and 15 days of each spraying, 30 compound leaves from 10 randomly selected plants/treatment were observed fer aphid counts as suggested by SIMPSON (1940) for evaluating the relative aphidicidal effect of insecticides. The pooled data were statistically analysed by following square root transformation $\sqrt{x+1}$ (SNEDECOR, 1956).

RESULTS AND DISCUSSION

The data on the population of green peach aphid, *Myzus persicae* Sulzer on potato under different treatments (pooled data for two years: 1976—1977 and 1977—1978) are presented in Table 1.

It is evident from aphid, *M. persicae* population data (Table 1) that all the six foliar systemic insecticides at both the dosages (300 and 500 g ai/ha/spraying) were statisticaly superior over control (water-spray) in reducing and keeping down the population of *M. persicae* on potato crop. Further, it was also observed that all these insecticides, in general, were equally effective at both the dosages. The trend of effectiveness of insecticidal treatments remained the same in all the observations (i. e. when aphid counts were taken on 5, 10 and 15 days interval after first and second spraying). On the basis of overall performance, the insecticides in their descending order of effectivity were oxydemeton-methyl > monocrotophos > thiometon > dimethoate > phosphamidon > formothion

Oxydemeton-methyl @ 300 and 500 g ai/ha/spraying were the most consistent treatments at all the times in reducing the population of aphid, *M. persicae* on on potato crop. This insecticide at its both the dosages was found to be the most effective for longer duration especially, when aphid population was high in check plots (360.76 aphids/10 plants). Besides, monocrotophos and dimethoate each @ 500 g ai/ha/spraying followed the oxydemeton-methyl (both the dosages) in giving the effective and long lasting control of *M. persicae*. As regards the duration of effectivity of these insecticides, they were ranked in their descending order of efficacy as oxydemeton-methyl > monocrotophos > thiometon > dimethoate > formothion > phosphamidon. Almost similar trend of efficacy of the foliar systemic insecticides against *M. persicae* has been reported by several other workers from various regions of the country. LAL & MISRA (1979) and MISRA *et al.* (1980), respectively, from Jalandhar (Punjab) and Patna (Bihar) have reported oxydemton-methyl and dimethoate as the best insecticides for the control *M. persicae* infesting potato crop. They have also observed that the foliar systemic insecticides were equally effective at 0.03 and 0.05% concentrations in controlling *M. persicae* on potato crop.

In conclusion, all the six foliar systemic insecticides viz. oxydemeton-methyl, monocrotophos, thiometon, phosphamidon, dimethoate and formothion

TABLE 1. The population of green peach aphid, *Myzus persicae* Sulzer on potato under different treatments (pooled data for two years: 1976–1977 & 1977–1978).

Treatments	Dose g a i ha per spraying	Average number of aphids/10 plants at 5, 10 and 15 days interval after first and second spraying					
		5 days		5 days		15 days	
		1st spraying	2nd spraying	1st spraying	2nd spraying	1st spraying	2nd spraying
Oxydemeton-methyl	300	1.00 (0.00)	1.48 (1.19)	1.33 (0.77)	1.14 (0.30)	1.07 (0.14)	2.80 (6.84)
—do—	500	1.00 (0.00)	1.33 (0.77)	1.07 (0.14)	1.14 (0.30)	1.00 (0.00)	2.44 (4.95)
Monocrotophos	300	1.00 (0.00)	1.38 (0.90)	1.00 (0.00)	3.33 (10.09)	1.14 (0.30)	5.17 (25.73)
—do—	500	1.00 (0.00)	1.62 (1.62)	1.24 (0.54)	2.34 (4.48)	1.07 (0.14)	3.54 (11.53)
Thiometon	300	1.07 (0.14)	2.19 (3.80)	1.53 (1.34)	3.22 (9.37)	1.43 (1.04)	5.69 (31.38)
—do—	500	1.00 (0.00)	1.47 (1.16)	1.24 (0.54)	3.71 (12.76)	1.72 (1.96)	5.12 (25.21)
Phosphamidon	300	1.07 (0.14)	2.25 (4.06)	1.19 (0.42)	4.38 (18.18)	1.73 (1.99)	5.92 (34.05)
—do—	500	1.00 (0.00)	2.12 (3.49)	1.24 (0.54)	4.43 (18.69)	1.69 (1.86)	5.61 (30.47)
Dimethoate	300	1.00 (0.00)	2.38 (4.66)	1.28 (0.64)	3.54 (11.53)	1.66 (1.76)	6.98 (47.72)
—do—	500	1.07 (0.14)	1.45 (1.10)	1.31 (0.72)	3.13 (9.11)	1.14 (0.30)	4.86 (22.62)
Formothion	300	1.14 (0.30)	2.87 (7.24)	1.31 (0.72)	2.75 (6.56)	1.61 (1.59)	6.88 (46.33)
—do—	500	1.07 (0.14)	2.41 (4.81)	1.14 (0.30)	3.69 (12.62)	1.42 (1.02)	5.92 (34.05)
Control (Water-spray)		2.34 (4.43)	8.74 (75.39)	3.65 (12.32)	17.43 (302.89)	4.79 (21.94)	19.02 (360.76)
S Em (±)		0.05	0.21	0.09	0.30	0.13	0.36
C D (0.05)		0.12	0.58	0.24	0.83	0.36	1.00

Figures in parentheses indicate retransformation in original units.

each @ 300 and 500 g ai/ha/spray treatment were found effective against *M. persicae* on potato crop in Deccan Plateau region of the country. Oxydemeton-methyl popularly known as metasystex 25 EC was relatively more effective insecticide for longer duration, especially, when aphid population was high. The insecticides at their lower dose (300 g ai/ha/spraying) were almost equally effective when compared with higher dose (500 g ai/ha/spray). Hence lower dose which has been found economically effective for aphid control on seed potato crop is recommended.

Oxydemeton-methyl could consistenly keep the population of *M. persicae* below the critical level (20 aphids/100 compound leaves) for a period of about 15 days even under situations when aphid population is high on seed crop of potato.

With a view to protecting potato crop, being grown for seed purposes, from *M. persicae* throughout the entire crop period with the help of foliar systemic insecticides alone, it is suggested that 3-5 sprayings with any of the foliar systemic insecticides, preferably with oxydemeton-methyl, may be given @ 300 g ai/ha/spraying, after diluting the insecticide in 1000 l of water. The aphid population in the region on early *rabi* and late *rabi* crops, which overlap with each other, remains quite high from the early stages of crop. Hence, first spraying may be given soon after the germination or sown before the arrival of aphids (i.e. in the first week of December) on the crop and subsequent 2-4 need-based sprayings may be given at fortnightly intervals.

Since oxydemeton-methyl and other systemic foliar insecticides are absorbed into the foliage and are translocated downward up to the tubers of sprayed crop because of their systemic action, a waiting period of six weeks between the last spray and harvest of the crop has been suggested (ANONYMOUS, 1971). According to AWASTHI *et al.* (1977) exeecssive residues of oxydemeton-methyl (above the tolerance limit of 0.2 ppm), resulted from 0.03% sprayable fluid, persisted upto 22-23 days following the last spray treatment on/in unwashed unpeeled as well as from unwashed but peeled potatoes. However processings viz. washing, boiling and peeling of potatoes eliminated any toxic residues of oxydemeton-methyl. Hence processings become a pre-requisite caution to be advocated, if potatoes grown for seed purpose are to be used for table purposes

Acknowledgement: The authors are grateful to Dr. B. B. NAGAICH, the then Director and to Dr. K. S. KRISHNA PRASAD, Head, Division of Entomology, Central Potato Research Institute, Shimla for their valuable suggestions. Assistance rendered by Shri C. V. DHANDHERE, Senior Field Assistant, Central Potato Research Station, Rajgurunagar, Pune is also acknowledged.

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Entomon, 1984, *Vol.* 10, *No.* 2, *pp.* 93 *to* 95

BRIEF COMMUNICATION

DENSITY OF POTENTIAL VECTOR OF DENGUE HAEMORRHAGIC FEVER, *AEDES AEGYPTI* (DIPTERA : CULICIDAE)

RAJINDER SINGH & KALPANA SHARMA

Department of Health, Urban Malaria Scheme, Gurgaon, Haryana, India 122 001

(*Received 31 October 1983*)

The density of Aedes aegypti in Gurgaon city was assessed from extensive surveys of the adult carried out from 2.5.83 to 15.10.83 to evaluate its role in the epidemiology of dengue haemorrhagic fever. All the areas where dengue haemorrhagic fever occurred, were surveyed. The results show that highest density of Aedes aegypti was recorded between 25.7.83 to 30.7.83. Temperature and relative humidity were quite consistent throughout the study.

(*Key words:* dengue, *Aedes aegypti*, density)

Dengue haemorrhagic fever is a problem of great public health importance in South-East Asia (RUDNICK, 1967). The most important vector, as confirmed wherever dengue fever occurs, is *Aedes aegppti*. Although *Aedes aegypti* is known to be widely distributed in several countries of South-East Asia, and its importance as a potential vector of dengue fever has been recognised for a long time, the information on its density and seasonal prevalance is still fragmentry. In India, a double peaked epidemic of dengue fever occurred in Calcutta in 1963 and 1964 (SARKAR *et al.*, 1964). Seasonal abundance of adult and immature *Aedes aegypti* was observed by NELSON *et al.* (1976). The distribution of *Aedes aegypti* at high elevations in Columbia was also studied by NELSON *et al.* (1983). Distribution, density and seasonal prevalance of *Aedes aegypti* in the Indian sub-continent and South-East Asia was studied by RAO (1967). Analysis of the mosquito collections in dengue fever infected localities was carried out by SINGH (1983). All evidence points to *Aedes aegypti* as the important vector of dengue haemorrhagic fever, this mosquito being the only species capable of transmitting dengue virus occurring in the crowded slums from where many of the cases are reported.

Because the incidence of dengue fever is usually highest during the rainy season, it is important to assess the seasonal fluctuation of the mosquito population for an understanding of epidemiology of the disease. The paper presents the findings on weekly density of *Aedes aegypti* in Gurgaon city.

Gurgaon city was divided into six sectors for entomological observations. All the six sectors in the city were surveyed between May 2, 1983 and October 15, 1983. These areas included all the densely populated parts of the city and represented the area where majority of dengue fever cases were reported in 1982 and 1983. Most houses are constructed of cement and some are

two storeyed. Houses with front and back-yards are common in single storey buildings under Haryana Urban Development Authority (HUDA). Protected water is supplied by Municipal Committee and HUDA. Most houses have their own water taps. It is only in some of the areas in the out-skirts that water has to be brought from distant public taps. Wells are extremely rare. The maximum rainfall was in the month of July, 1983 (476.5 mm).

The mosquitoes landing on human bait or resting on nearby objects were collected weekly in five permanent stations in each sector by one insect collector between 0600 to 0800 hr spending fifteen minutes per station. Collections were also made from eighteen random capturing stations from peripheral and central zones of the city. One insect collector visited eight houses for fifteen minutes per house. This resulted in a sample of 2 man-hours for each collection day.

The adult density for *Aedes aegypti* are given in Table 1. No consistent seasonal pattern of population density

TABLE 1. Weekly change in *Aedes aegypti* density in Gurgaon city.

Week (1983)	Time spent (Hours)	Density per man hour	Rainfall (mm)
2.5 to 7.5	12	1.1	
9.5 to 14.5	12	1.3	85.5
16.5 to 21.5	8	0.4	
23.5 to 28.5	12	1.3	
30.5 to 4.6	10	0.4	
6.6 to 11.6	10	5	54.5
13.6 to 18.6	10	0.8	
20.6 to 25.6	10	0.7	
27.6 to 2.7	12	0.7	
4.7 to 9.7	12	0.7	
11.7 to 16.7	8	0.5	476.5
18.7 to 23.7	12	1.2	
25.7 to 30.7	10	3.7	
1.8 to 6.8	8	3	
8.8 to 13.8	10	3.5	44.5
15.8 to 20.8	4	0.5	
22.8 to 27.8	12	2.6	
29.8 to 3.9	Collection was not made		
5.9 to 10.9	Collection was not made		178.5
12.9 to 17.9	12	1.6	
19.9 to 24.9	10	3.4	
26.9 to 1.10	12	2.8	
3.10 to 8.10	12	3	2
10.10 to 15.10	10	1.9	

was evident in the city. The apparent peaks of *Aedes aegypti* density between 25.7.1983 to 30.7.1983 corresponded to rainfall. In July, 1983 there was heavy rainfall (476.5 mm) and the peak density of *Aedes aegypti* (3.7, 3 & 3.5/man hr) was recorded in the weeks of 25.7 to 30.7.1983., 1.8 to 6.8.1983 and 8.8 to 13.8.1983 respectively. Both the breeding index and density of *Aedes aegypti* are affected by temperature and rainfall (SOPER, 1967). In other countries in South-East Asia several similar type of studies have tended to relate the density of *Aedes aegypti* to weather, season and incidence of dengue fever (SCANLON, 1966). In Singapore during 1966—1968 the density of adult *Aedes aegypti* from the in-door resting collections generally fluctuated with rainfall (HO *et al.*, 1971) and with dengue fever incidence (CHAN *et al.*, 1971). Haemorrhagic fever in South-East Asia and India has been one of the primary reasons for the new alert to the increased dangers presented by *Aedes aegypti* (RUDNICK, 1967). Epidemics of dengue diseases caused by dengue virus recently in October and November, 1982 in the Gurgaon city, all apparently transmitted by *Aedes aegypti* (SINGH, 1983). During this study it was clear that *Aedes aegypti* density is directly correlated with rainfall as recorded by SCANLON (1966).

Acknowledgements: Authors are thankful to SH. JAIVIR SHARMA, Superior Field Worker and SH. OMPARKASH, Insect collector for their generous help in the collection of entomological data. Authors are also very grateful to Dr. K. L. SIKKA, Joint Director (Malaria) for guidance.

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Entomon, 1984, *Vol.* 10, *No.* 2, *pp.* 97 *to* 106

CHROMOSOMES OF SEVEN SPECIES OF INDIAN GRASSHOPPERS

S. K. DEY, A. RAI & M. AGARWAL

Cytogenetics Laboratory, P. G. Department of Zoology, Darjeeling Govt. College, Darjeeling, W. Bengal, India 734 101

(*Received 17 October 1983*)

Meiotic chromosomes of seven species of grasshoppers viz, *X. humilis humilis*, *S. splendeus*, *S. prasiniferum prasiniferum*, *C. cachara* (have 2n ♂ = 23) and *gastrimargus* sp. (2n ♀ = 24) of the family Acrididae; *H. indica* (2n ♂ = 29) and *M. elongata* (2n ♀ = 28) of the family Tettigonidae have been studied. All the species are characterized by acrocentric chromosomes except *M. elongata*, which has two pairs of meta- or submetacentric chromosomes. The sex determining mechanism is XO in males and XX in females. Chiasma frequency and terminalization coefficient studies have been made in detail. Some chromosomal abnormalities such as anaphase bridges, supernumerary chromosomes and laggard are also seen. Metrical study from second division metaphase chromosomes has been made, and the role of chromosomal rearrangements in the karyotype evolution in these species of grasshoppers has been discussed.

(*Key words:* chromosomes, meiosis, grasshoppers)

INTRODUCTION

Our present knowledge on the chromosomes of Indian Acrididae and Tettigoniidae are very limited in comparison to other parts of the world (MAKINO, 1956; WHITE 1973). The cytological works on Indian grasshoppers have largely been carried out by ASANA (1934); ASANA *et al.* (1938, 1939); RAO (1937); RAY-CHAUDHURY & DUTT (1947); RAY-CHAUDHURI & MANNA (1951); RAY-CHAUDHURY & GUHA (1952); MANNA (1954); DUTT (1955); CHATTERJEE *et al.* (1971); CHATTERJEE & MANNA (1971); SHARMA *et al.* (1974). The present paper incorporates an account of the structure and behaviour of chromosomes during meiosis in five species of short-horned and two species of long-horned grasshoppers, which is a part of our cytological survey on the grasshopper fauna of Eastern Himalayan regions.

MATERIALS AND METHODS

The following species of short-horned grassphoppers constitute the materials for the present study: Family: Acrididae, *Xenocatantops humilis humilis* (Serville); *Stenocatantops splendeus* (Thunberg); *Spathosternum prasiniferum prasiniferum* (Walker); *Caryanda cachara* (Kirby); *Gastrimargus* sp.; Family: Tettigoniidae, *Holochlora indica* Linnaeus; *Mecapoda elongata* Kirby. All individuals were collected from Darjeeling and adjoining hill areas during June to Augugt 1982. The testes of five species and ovary of two species were fixed in 1:3 acetic-alcohol mixture. The fixed tissue was put into 45% acetic acid for a minute and then squashed between a cover glass and a slide smeared with Mayer's albumin and dried before use. After squashing by the thumb pressure under cover of a blotting paper, the slide was dried periodically under a table lamp for about 30 minutes. The slide was then immeresed into a jar of 50% alcohol overnight for the detachment of the cover glass. It was then passed through grades of alcohol to water and mordanted

in 3% iron-alum solutiou, stained in 1% Heidenhain's haematoxylin and differentiated in saturated aqueous solution of picric acid. The slide was thoroughly washed in running water, dehydrated in alcohol, cleared in xylene and mounted in DPX. Temporary squash preparation was also made by staining the fixed tissue in 2% lacto-acetic orcein. The chromosome measurement was made from the 10 second division metaphase plates, and the chiasma frequency was studied from 20 diplotene, diakinesis and metaphase I plates respectively.

RESULTS

Family : Acrididae

Xenocatantops humilis humilis (Serville)

Spermatogonial metaphase plates show 23 acrocentric chromosomes (Fig. 1). The general course of meiosis is typical acridid type and the *X*-chromosome remains positively heteropycnotic up to diakinesis stage. At metaphase I. the autosomal bivalents are arranged in an equatorial plane while the *X*-chromosome is laying at various positions of the spindle near the equatorial region (Fig. 2).

A study of chiasma frequency reveals that the loss of chiasmata is stronger between diakinesis and metaphase I (Table 1). The analysis of the data on the measurement of the second division metaphase chromosomes (Table 2) reveals that there are six long, five medium, and one short size chromosomes. However, the size difference between the different groups is not very appreciable. The *X*-chromosome belongs to long size group and second in order of length.

Stenocatantops splendeus (Thunberg)

The diploid set of chromosome consists of 23 acrocentric chromosomes. The general pattern of meiosis is quite typical. At metaphase II stage, the chromatids diverge widely, being only held together at the centromere (Fig. 3).

A study of chiasma frequency reveals that the loss of chiasmata is more pronounced between diakinesis and metaphase I as found in *X. humilis humilis*. Moreover, the terminalization coefficient and mean chiasma frequency per nucleus is more or less similar in both the species (Table 1). At diplotene stage, the number of chiasmata in the longest bivalent never exceed three. An analysis of the metrical data, however, shows that the chromosomes of *S. splendeus* are smaller than those of *X. humilis humilis* (Table 2). The chromosomes may be classified into two long, nine medium and one short size chromosomes. The *X*-chromosome belongs to medium size group and third in order of length.

Spathosternum prasiniferum prasiniferum (Walker)

Spermatogonial metaphase plates reveal the presence of 23 acrocentric chromosomes. It has also been found that the diploid chromosome number and general course of meiosis is agreeable with those of *S. prasiniferum* reported earlier by ASANA *et al.* (1939). However, in some of the diakinesis (Fig. 4) and metaphase I (Fig. 5) stages, an extra chromosome is encountered. But individuals having supernumerary chromosomes in all the dividing cells are not obtained.

A study of chiasma frequency reveals that there is an appreciable loss of chiasmata between diakinesis and metaphase I (Table 1). In the diplotene stage of meiosis, the number of chiasmata in the longest bivalent never exceed three, whereas the shorter bivalent have only

TABLE 1. Mean chiasma frequencies and terminalization coefficients at the different stages of meiosis in four species of short-horned grasshoppers.

Species	Stage	No. of nuclei	Total chiasmata	Total terminal chiasmata	Terminalization coefficient	Mean chiasmal frequency per nucleus ± SE of the mean
X. humilis humilis	Diplotene	20	249	49	0.19	12.4 ± 0.18
	Diakinesis	20	231	55	0.23	11.5 ± 0.15
	Metaphase I	20	212	90	0.42	10.6 ± 0.02
S. splendens	Diplotene	20	235	38	0.16	12.0 ± 0.32
	Diakinesis	20	227	46	0.20	11.3 ± 0.55
	Metaphase I	20	183	87	0.47	9.1 ± 0.64
S. prasiniferum prasiniferum	Diplotene	20	271	60	0.22	13.5 ± 0.74
	Diakinesis	20	216	109	0.50	10.8 ± 0.14
	Mataphase I	20	120	98	0.81	6.0 ± 0.07
C. cachara	Diplotene	20	260	41	0.15	13.0 ± 0.18
	Diakinesis	20	243	57	0.23	12.1 ± 0.26
	Metaphase I	20	165	74	0.44	8.2 ± 0.58

one chiasma each. A metrical study indicates that the chromosomes can be classified into five long, five medium and two short size chromosomes. The *X*-chromosome belongs to long size group and third in order of length (Table 2). The mean lengths of the chromosomes are found to be more or less similar with those of *X. humilis humilis* except *X*-chromosome, which is comparatively shorter than that of *X. humilis humilis* (Table 2).

Caryanda cachara (Kirby).

Spermatogonial metaphase plates show the presence of 23 acrocentric chromosomes. Meiosis is normal and orthodox. At metaphase I, the autosomal bivalents are arranged in an equatorial plane while the *X*-chromosome usually lies at various places of the spindle near the equatorial region (Fig. 6). On some rare occasions, sticky bridges at anaphase II are observed (Fig. 7). The maximum number of chiasmata observed in a diplotene bivalent is four.

The chiasma frequency study reveals that the loss of chiasmata is frequent between diakinesis and metaphase I (Table 1). The terminalization coefficient and mean chiasma frequency per nucleus found to be more or less similar with those of *X. humilis humilis* and *S. splendeus*, but differ with those of *S. prasiniferum prasiniferum* (Table 1). The analysis of the data on the measurement of second division metaphase chromosomes reveals that there are nine long, two medium and one short size chromosomes. The *X*-chromosome is a

TABLE 2. Mean lengths and percentage lengths of the second division metaphase chromosomes in four species of short-horned grasshoppers.

Species		Chromosome Numbers 1	2	3	4	5	6	7	8	9	10	11	X
X. humilis humilis	Mean length in μm	6.85 ±.10	5.95 ±.10	5.20 ±.11	4.40 ±.06	4.05 ±.08	3.80 ± 06	3.65 ±.10	3.20 ± 11	2.45 ±.09	2.10 ± 14	1.55 ± 13	6.1 ±.10
	% length	13.89	12. 0	10.54	8.92	8.21	7.70	7.40	6.49	4.90	4.25	3.14	12.37
S. splendens	Mean length in μm	5.76 ±.09	4.08 ±.15	3.53 ±.13	3.04 ±.19	3.07 ±.18	2.70 ±.15	2.60 ±.14	2 33 ±.13	2.18 ±.18	2.08 ±.09	1 47 ±.09	3.1 ±.23
	% length	16.02	11.35	9.82	8.45	8.54	7.51	7 23	6.48	6 06	5.78	4.09	8.62
S. prasinifernn prasinifernm.	Mean length in μm	6.95 ±.50	5.60 ±.23	4.70 ± 2)	4.25 ±.20	3.90 ±.20	3.50 ±.18	3.50 ±.13	3.00 ± 17	3.00 ±.16	2 20 ±.13	1.70 ±.12	5.0 ±.07
	% length	14.69	11.83	9.93	8.98	8.24	7.39	7.39	6.34	6.34	4.65	3.59	10.57
C. cachara	Mean length in μm	14.51 ±.36	12.53 ±.44	10.82 ±.55	9.71 ±.36	8.56 ±.12	7.49 ± 22	6.70 ±.19	6.22 ± 24	4 28 ±.15	3.37 ±.26	2.37 ±.11	12.04 ±.80
	% length	14.71	12.70	10.97	9.84	8.68	7.59	6.79	6.30	4.34	3.41	2.40	12.21

± SEM

member of the long size class and third in order of length (Table 2). However, it has been found that the actual length of the chromosomes of this species is widely different from other members of Acrididae under present investigation. But when their percentage lengths are compared, they show more or less striking similarities. Therefore, it is possible that the difference in their actual chromosome length may be due to the differential spiralization.

Gastrimargus sp.

The diploid chromosome number in this species is determined from female individuals only. The oogonial metaphase plates reveal the presence og 24 acrocentric chromosomes (Fig. 8). An analysis of the metaphase chromosome shows that there are eight long, twelve medium and four short chromosomes. The sex chromosome pair is not recognizable from the autosomes. In spite of our best effort, no male individuals are found for study of the meiosis.

Family: Tettigoniidae

Holochlora indica Kirby

The spermatogonial metaphase plates consists of 29 acrocentric chromosomes (Fig. 9) of which the *X*-chromosome is the longest of all. Besides the extraordinarily large *X*-chromosome, first two pairs of autosomes can also be demarcated from the remaining gradually seriated medium to short size autosomes. Thus the karyotype of *H. indica* found to be more or less similar with that of *L. punctipe, D. japonica* and *I. stylata* reported by CHATTERJEE & MANNA (1971).

In the early prophase stage, *X*-chromosome can be recognized as deeply stained body among diffusely stained autosomes (Fig. 10). At diakinesis (Fig. 11) and metaphase I (Fig. 12) stages, 14 bivalents are regularly formed by 28 autosomes while the long univalent *X*-chromosome can clearly be demarcated from them. However, it is interesting to mention that the longest *X*-chromosome often forms sticky bridges (Fig. 13) before separating normally to opposite poles (Fig. 14) during anaphase II stages. On some rare occasions, laggards formed by sex chromosome are also observed (Fig. 15). Due to inadequate number of divisional stages, metrical and chiasma frequency studies are not possible.

Mecapoda elongata Linnaeus

The diploid chromosome number is determined from a female individual. The oogonial complement contains 28 chromosomes (Fig. 16), which consist of 6 large, 4 medium and 18 small chromosomes. All the chromosomes are acrocentric, except two pairs of large chromosomes which are meta or submetcentric in nature. The sex chromosome pair cannot be identified from autosomes. Oogonial anaphase (Fig. 17) also shows two pairs of 'V' shaped meta- or submetacentrics and rod shaped acrocentric chromosomes. Unfortunately, no male individuals are found for study of the meiosis.

DISCUSSION

An apparent karyotypic uniformity which is observed in majority of grasshopper species belonging to the large family Acrididae, is also found in all the species under present investigation. The diploid chromosome number is 23 (22A+XO) in four male specimens and 24 (22A + XX) in a female specimen.

The course of meiosis is normal and orthodox. The chiasma study shows that in all the four species the loss of chiasmata is more frequent between diakinesis and metaphase I. However, when the chiasma frequency of *X. humilis, humilis* and *L. splendeus* are compared with that of another closely related species *C. humilis* (MANNA, 1954) shows that in the latter the loss of chiasmata is frequent between diplotene and diakinesis stages. Further, the comparison of the metrical data reveals that the total complementary length of the chromosomes of *C. humilis* is more or less similar with that of *S. splendeus,* but varies appreciably from that of *X. humilis humilis.*

When the relative lengths of the second division metaphase chromosomes of the four species are plotted graphically (Fig. 18), it becomes evident that most of the chromosomes have undergone marked re-arrangements in the course of evolution of different species. Among the autosomes, the 1st, 3rd, 5th and 9th are most variable ones, the rest varying to a comparatively lesser extent. The 2nd and 3rd autosomes of *S. splendeus* are smaller than those of other species, while the 1st is considerably longer than that of the other species. It is likely, therefore, that some interchromosomal changes might have taken place between these chromosomes in *S. splendeus.*

The *X*-chromosome in *S. splendeus* is 8.62% and in *X. humilis humilis* it is 12.37%. The measurements of the *X*-chromosome of other species lie within this range (see Table 2). It is, therefore, highly probable that more change has taken place in this particular chromosome (see Fig. 18) than in any other autosome during evolution of the different species.

In the family Tettigoniidae the spermatogonial chromosome number ranges from 20 to 35 (WHITE, 1973). Moreover, the kariotypic conservation which is observed in the grasshoppers of the family Acrididae, is not found among the members of the family Tettigoniidae. The diploid chromosome number in one male and one female specimens under present investigation have 29 and 28 chromosomes respectively. CHATTERJEE & MANNA (2971) also reported the presence of 29 acrocentric chromosomes in three male specimens of Indian Tettigoniidae. According to WHITE (1973),

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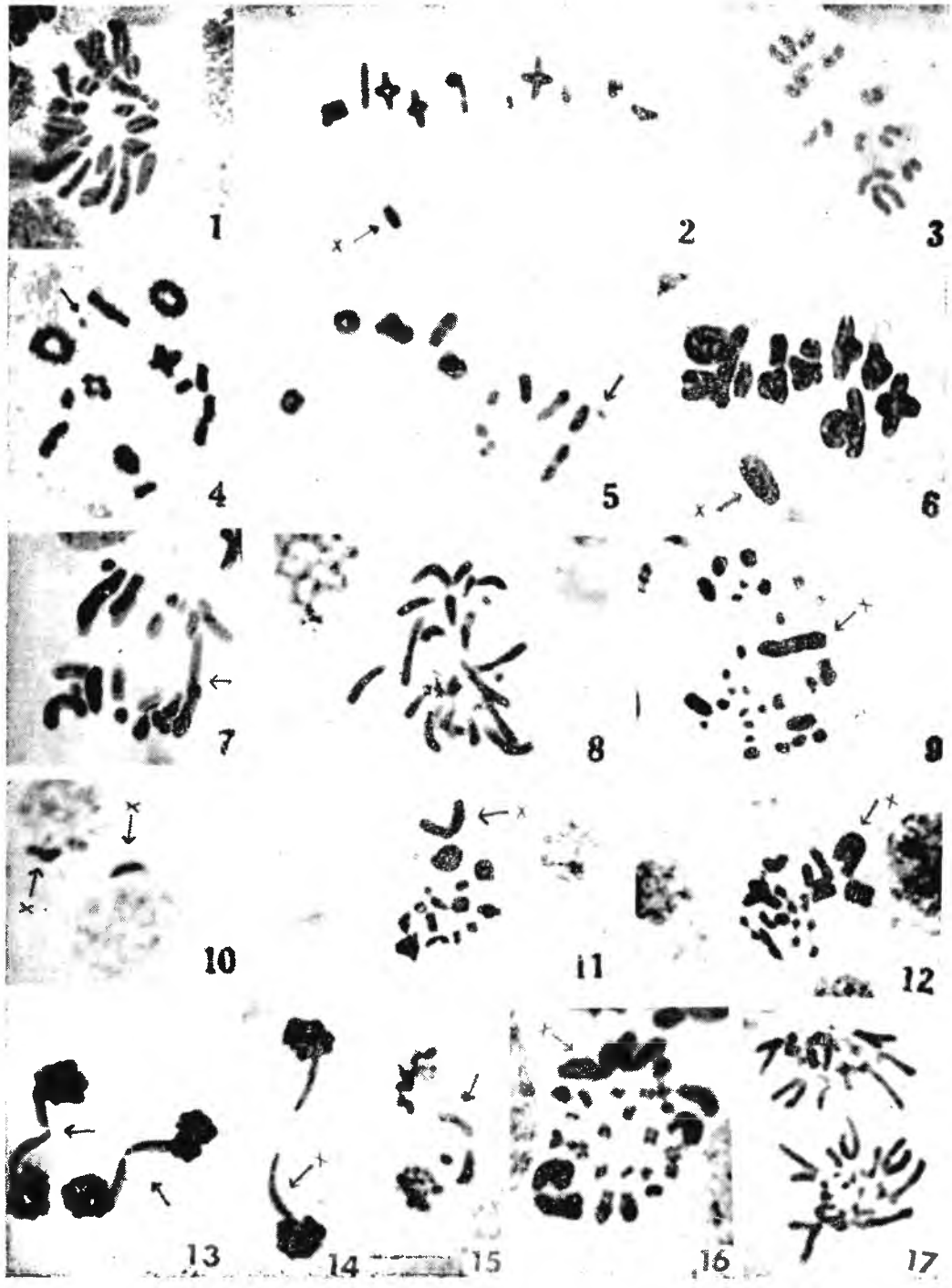
Photomicrographs of grasshoppers (× 1000). Fig. 1. Spermatogonial metaphase of *X. humilis humilis* showing 23 chromosomes; Fig. 2. Metaphase I of *X. humilis humilis* showing 12 elements; Fig. 3. Metaphase II of *S. splendeus* showing 12 elements; Fig. 4. Diakinesis of *S. prasiniferum prasiniferum* showing an extra chromosome; Fig. 5. Metaphase I of *S. prasiniferum prasiniferum* showing an extra chromosome; Fig. 6. Metaphase I of *C. cachara* showing 12 elements; Fig. 7. Anaphase II with a sticky bridge in *C. cachara*; Fig. 8. Oogonial metaphase of *Gastrimargus* sp. showing 24 chromosomes; Fig. 9. Spermatogonial metaphase of *H. indica* showing 29 chromosomes; Fig. 10. Two early propoase I stages in *H. indica* showing positively heteropycnotic *X*-chromosomes; Fig. 11. Diakinesis of *H. indica* showing 15 elements; Fig. 12. Metaphase I of *H. indica* showing 15 elements; Fig. 13. Two late anaphase stages with sticky bridges in *H. indica*; Fig. 14. Late anaphase showing normal separation of *X*-chromosomes in *H. indica*; Fig. 15. Late anaphase showing laggards in *H. indica*; Fig. 16. Oogonial metaphase showing 23 chromosomes in *M. elongata*; Fig. 17. Oogonial anaphase showing rod soaped and 'V' shaped chromosomes in *M. elongata.*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the species of Tettigoniidae with relatively low chromosome numbers have several pairs of metacentric chromosomes which have arisen by centric fusion. Similar interchromosomal rearrangement

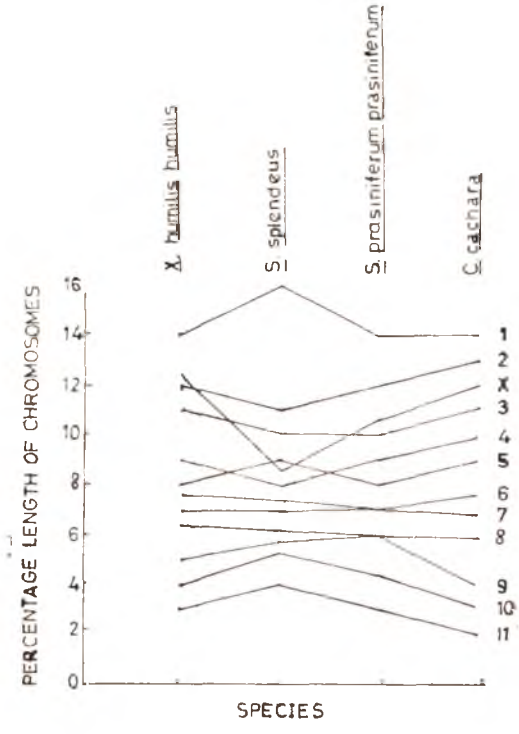

Fig. 18. Percentage length of chromosomes in four species of grasshoppers. 1—11 on the right represent the 11 autosomes in order of length; X = sex chromosome.

may be responsible for the occurrence of meta- or submetacentric chromosomes in *M. elongata*

Acknowledgements: The authors are grateful to the Zoological Survey of India, Calcutta, for the identification of specimens and Head Department of Zoology, for providing laboratory facilities. The authors are indebted to Prof. G. K. MANNA Department of Zoology, University of Kalyani and Dr. B. DASGUPTA, Principal Darjeeling Govt. College for encouragement. Thanks are due to Mrs. K. DEY for helping in various ways.

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Entomon, 1984, *Vol.* 9, *No.* 2, *pp.* 107 *to* 113

STUDIES ON INDIAN SPECIES OF *DICAMPTUS* SZEPLIGETI (HYMENOPTERA : ICHNEUMONIDAE)

P. K. NIKAM & L. J. KANHEKAR

Department of Zoology, Marathwada University, Aurangabad, India 431 004

(Received 13 August 1983)

Two new species of *Dicamptus* Szepligeti from India viz. *D. bicarinus*, and *D. lambai* are described and *D. nigropictus* (Matsumura) is recorded for the first time as Indian. A key to the Indian species of *Dicamptus* is provided.

(Key words: Dicamptus species of India)

Dicamptus Szépligeti (1905) is a moderate sized genus occurring in the Oriental, Australian and Ethiopian regions (Townes, 1971). Townes, Townes and Gupta (1961) included eleven species of this genus in the Indo-Australian region. Nikam (1972) added one more species from India. Gauld (1977) placed Australian *D. conspicuus* (Morley) and *D. emandibulator* (Morley) under *Riekophion* Gauld and *D. bakeri* (Cheesman) under *Leptophion* Cameron. So far only two species, *D. reticulatus* (Cameron, 1899) and *D. indicus* Nikam were known from India. Two new species, *D. bicarinus* and *D. lambai* from India are described and *D. nigropictus* (Matsumura, 1912) is recorded for the first time as Indian. A key to the Indian species of *Dicamptus* is also included.

Identifications of the species were confirmed during the stay of the senior author at the British Museum (Natural History), London and the material examined was from the collection of:

PKN : P. K. Nikam Collection, Department of Zoology, Marathwada University, Aurangabad, India.

ZSI : Zoological Survey of India, Culcutta, India.

The terminology used in this work follows that of Richards (1956) as interpreted for the Ophioninae by Gauld (1977), Gauld and Mitchell (1978) and Nikam (1980). The figures were drawn with camera lucida.

1. Dicamptus bicarinus, sp. nov.
(Figs. 1—5)

Mandible without a diagonal groove, outer surface with scatter pubescence, medially 0.60 times as broad as at base, teeth equal; malar space 0.30 times the basal mandibular width; lower face 0.85 times as broad as long; face convex, finally, sparsely pubescent, rugulose; clypeus finely, sparsely pubescent, somewhat nose-like, rugose, apically transverse, clypeofacial suture distinct; posterior ocelli separated from eyes by 0.33 times their diameter; 1st flagellar segment 1.30—1.40 times as long as 2nd segment and 20th segment 2.50 times as long as broad; occipital carina strong, occipital width 1.95 times the maximum diameter of posterior ocellus.

Mesoscutum in profile evenly rounded, sparsely, finely punctate, notaulus slightly impressed in basal half; scutellum shiny, basally very finely, sparsely, shallowly punctate, apically with 2 longitudinal

median carinae, propleurum shiny, longitudinally striate; mesopleurum striatopunctate to punctate, epicnemial carinae curved, not reaching the anterior margin of the pleuron; metapleurum longitudinally striate, intermixed with punctae; propodeum finely reticulate, with anterior transcarina, spiracle semicircular, without a distinct carina joining rim of the spiracle to pleural carina. Fore wing 6.80 mm in length; AI = 1.00; CI = 0.20; DI = 0.35; ICI = 0.25; *Rs* + 2*r* elbowed, thicker; *Rs* 1.60 times as long as *Rs* + 2*r*; *M* between 2*m*—*cu* and 3*rm* curved; 1*m*—*cu* evenly curved, apically fenestrate; *cu*—*a* slightly proximaly to *Rs* & *M*; 1st abscissa of Cu_{1a} 0.90 times as long as the 1st abscissa of Cu_1; 3 *rm* 0.50 the length of 2*m*—*cu*; fenestra with 2 sclerites, proximal sclerite strongly sclerotized, distal sclerite moderately sclerotized, elongate, concave towards proximal sclerite; hind wing with 2 + 5 hamuli on R_1; NI = 2.00; basal abscissa of *Rs* straight. Outer surface of foretibia with sparse, long, downcurved spines; hind coxa 1.70 times as long as deep, trochantellus mediodorsally 0.55 times as broad as long, longer tibial spur 1.60 times as long as shorter spur, claw pectinate.

Gaster slender, pubescent; 1st tergite as long as 2nd, with small circular spiracle at 0.65; 3rd tergite 1.40 times as long as broad:

Male: Unknown

Brown. Frons, face pale yellow; proximal sclerite blackish; veins yellowish-brown; interocellar area, eyes mandibular teeth, gaster terminally black.

Holotype: ♀ INDIA: MAHARASHTRA, OMERGA (Osmanabad Dtstrict) 2.x.1975, 1 ♀ coll. K. S. Heble (PKN).

Remarks: This species is very characteristic, differs from all Indian species of *Dicamptus* in having somewhat nose-like clypeus and distal sclerite elongate, concave toward proximal sclerite.

Distribution: India: Maharashtra.

2. Dicamptus lambai, sp. nov. (Figs. 6—9)

Mandible without a diagonal groove, outer surface with scatter pubescence, medially 0.75 times as broad as at base, teeth equal; malar space 0.30 times the basal mandibular width; lower face 0.80 times as broad as long; face convex, pubescent, densely punctate; clypeus convex, sparsely punctate, sparsely pubscent, apically transverse, clypeo-facial suture very distinct; posterior ocelli separated from eyes by 0.08 times their diameter; 1st flagellar segment 1.65 times as long as 2nd segment, 20th segment 2 times as long as broad; occipital carina strong, occipital width 1.80 times the maximum diameter of posterior ocellus.

Mesoscutum in profile evenly rounded, finely punctate, pubescent, notaulus weakly impressed; scutellum shiny, basally sparsely, finely punctate, apically longitudinally striate; propleurum striatopunctate; mesopleurum punctate to striatopunctate, epicnemial carina weakly curved, not reaching to the anterior margin of pleuron; metapleurum rugosopunctate; propodeum distinctly rugosoreticulate, with anterior transcarina; spiracle elongate, without a distinct carina joining rim of the spiracle to pleural carina. Fore wing 16.25 mm, in length; AI = 0.85; CI = 0.75; DI = 0.42; ICI = 0.65; *Rs* + 2*r* centrally sinuous, thicker; *Rs* 1.75 times as long as *Rs* + 2*r*; *M* between 2*m*—*cu* and 3*rm* curved; 1*m*—*cu* sinuate, apically fenestrate; *cu*—*a* slightly proximal to *Rs* & *M*; 1st abscissa of Cu_{1a} 1.65 times as long as 1st abscissa of Cu_1; 3*rm* 0.65 the length of 2*m*—*cu*; fenestra with 2 sclerites, proximal sclerite moderately sclerotized, large broadly

subtriangular, appendiculate, distal sclerite very weakly pigmented, small; hind wing with 2 + 8 hamuli on R_1; NI = 2.35, basal abscissa of *Rs* straight; distal abscissa of *Rs* with basal stub, rest weakly pigmented. Outer surface of fore tibia and basitarsus with long numerous downcurved spines; hind coxa 1.80 times as long as deep, trochantellus mediodorsally 0.65 times as broad as long, longer tibial spur 1.30 times as long as shorter spur, claw pectinate, with 1 + 11 teeth and 2 short, stiff, immobile hairs.

Gaster slender, pubescent; 1st tergite with a small circular spiracle at 0.67; 2nd terigite 0.95 times as long as 1st tergite; 3rd tergite 1.40 times as long as broad.

Female: Unknown.

Brown. Frons, face, clypeus pale brown; interocellar area, eyes, veins, mesoscutum except dorsolateral and median portions, scutellum dark brown; 3rd tergite medially, gaster terminally dark brown to blackish-brown

Holotype: ♂, INDIA: UTTAR PRADESH Ghat, Garhwal Dist, alt. 38 ft., 17. viii 1958. Coll. B. S. Lamba (ZSI).

The species is named after the collector, B. S. Lamba.

Remarks: This species is characterized by having AI = 0.85, 1st abscissa of Cu_{1a} 1.65 times as long as 1st abscissa of Cu_1 and distal abscissa of *Rs* in hind wing with a basal stub.

Distribution: INDIA: Uttar Pradesh.

3. Dicamptus nigropictus (Matsumura) (Figs. 10—13).

Ophion nigropictus Kuroiwa, 1908, Provisional List of the Hymenoptera collected in Loochoo determined by Dr. Matsumura, p. 1. *Nomed nudum*; Matsumura, 1912, Thousand insects of Japan, Supplement 4 : 113.

Henicospilus nigropictus Matsumura & Uchida, 1926, *Insecta matsum.* 1 : 71.

Dicamputs nigropictus Uchida, 1928, *J. Fac. Agric. Hokkedo (imp.) univ* 21 : 211.

Dicamptus nigropictus var. *fuscus* Uchida, 1928, *J. Fac. Agric. Hokkaido (imp.) Univ.* 21 : 211. (Synonymized by Townes, Townes and Gupta, 1961, p. 267).

Dicamptus nigropictus Matsumura, 1930 Illustrated Thousand Insects of Japan 2, (English section); 41; (Japanese section); 119; Matsumura, 1931, 6000 Illustration of insects of Japan p. 43; *Chiu,* 1935, *Yb. Bur. Ent. Hangchow,* 1934, 14.

Enicospilus flavoplagiatus Cushman, 1937, *Indian Forest Rec.* (n. s. Ent.) 3 : 144.

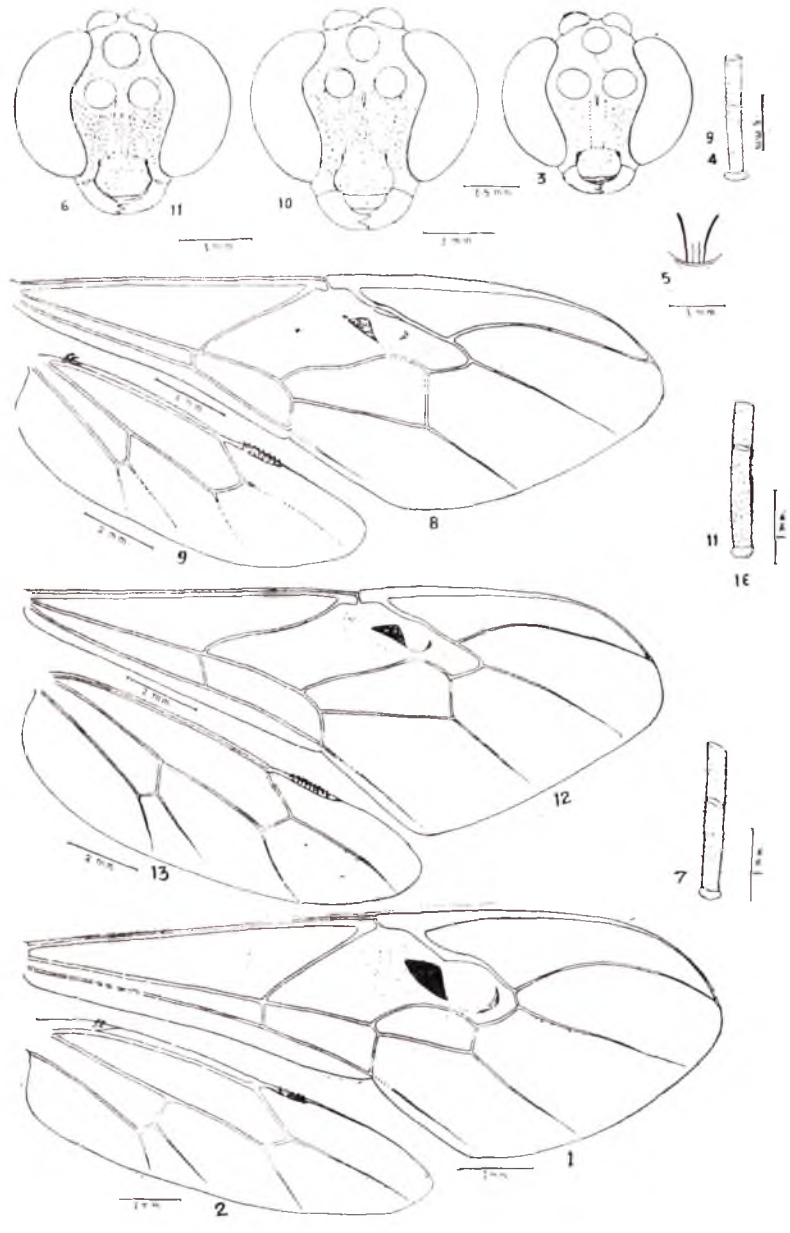
Enicospilus nigropictus Chiu, 1954, *Bull. Taiwan agric. Res. Inst.*, 13:47

Enicospilus uchidae Chiu, 1954, *Bull. Taiwan agric. Res. Inst.*, 13:49.

Dicamptus nigropictus Townes and Gupta, 1961, *Mem. Amer. Ent. Inst.*, 1:267.

Mandible without a diagonal groove outer surface with sparse pubescence, medially 0.60—0.65 times as broad as at base, teeth equal; malar space 0.14—0.15 times basal mandibular width; lower face 0.80—0.90 times as broad as long; face convex, sparsely pubescent closely punctate; clypeus convex, sparsely punctate, sparsely pubescent, apically weakly emarginate, clypeo-facial suture indistinct; posterior ocelli just touching to eyes; 1st flagellar segment 2—2.15 times as long as 2nd, 20th segment 1.80—1.85 times as long as broad; occipital carina strong, occipital width 1.50—1.70 times the diameter of posterior ocellus.

Mesoscutum in profile evenly rounded, finely punctate, pubescent, notaulus weakly impressed; scutellum shiny, basally



Dicamptus bicarinus sp. nov. (Figs. 1—5). 1. Fore wing; 2. Hind wing; 3. Head viewed from front; 4. First two flagellar segments; 5. Scutellum with carinae; *Dicamptus lambai* sp. nov. (Figs. 6—9). 6. Head viewed from front; 7. first two flagellar segments; 8. front wing; 9. Hind wing; *Dicamptus nigropictus* (Matsumura) (Figs. 10—13). 10. Head viewed from front; 11. First two flagellar segments; 12. front wing; 13. Hind wing.

sparsely, finely punctate, apically with strong striate; propleurum rugulose; mesopleurum basally punctate, rest rugosopunctate to striatopunctate: epichemial carina curved, not reaching the anterior margin of pleuron: metapleurum striatopunctate to rugoso-punctate; propodeum strongly reticulate, with an anterior transcarina, spiracle elongate, without a distinct carina joining rim of the spiracle to pleural carina. Fore wing 15.30—20.30 mm. in length; AI = 1.22—1.16; CI = 0.68—0.80; DI = 0.40—0.45; ICI = 0.70—0.85; *Rs* + 2*r* centrally sinuous, thicker; *Rs* 2—2.25 times as long as *Rs* + 2*r*; *M* between 2*m*—*cu* and 3*rm* curved; 1*m*—*cu* sinuate and apically fenestrate; *Cu*—*a* proximal to *Rs* & M; 1st abscissa of Cu_{1a} 1.10—1.50 times as long as 1st abscissa of Cu_1; 3*rm* 0.75—0.85 the length of 2*m*—*cu*; with 2 sclerites, proximal sclerite moderately sclerotized, large, subtriangular, distal sclerite pigmented, small; hind wing with 9—10 hamuli on R_1; NI = 1.80—2; basal; abscissa of *Rs* weakly curved at base; distal abscissa of *Rs* pigmented throughout. Outer surface of fore tibia and basitarsus with numerous long downcurved spines; hind coxa 2 times as long as deep, trochantellus mediodorsally 0.55—0.60 times as long ae broad, longer tibial spur 1.45 times as long as shorter spur, claw pectinate with 1 + 9 teeth and 2 short, stiff, immobile hairs.

Gaster moderately long, pubescent; 1st tergite as long 2nd, with a small circular spiracle at 0.67; 3rd tergite 1.28—1.30 times as long as broad; ovipositor exerted, 0.70—0.85 as long as apical depth of abdomen.

Male: Unknown

Dark brown. Ocellar area, eyes, gaster terminally blackishbrown; ocelli, thorax blackish; mandibular teeth black; ovipositor reddish-brown.

Material examined: INDIA: UTTAR PRADESH, Birahi, Garhwal Dist; alt. 7000 ft., 16.viii. 1958, 1 ♀, Coll. B. S. Lamba (ZSI); INDIA: ARUNACHAL PRADESH, Kameng, Dirong Dzong, Milankhang Valley, alt. 2135 m., 7.viii. 1961, 1 ♀, Coll. S. Biswas (ZSI).

Remarks: This species resembles *D. reticulatus* (Cameron) in the characters of clypeo-facial suture, vertex, foretibia, basitarsus, *Rs* + 2*r* and distal abscissa of *Rs* in hind wing. However, it differs from the same in having mandible without a diagonal groove, sparse pubescence on outer surface: propodeum without a distinct carina joining rim of the spiracle to pleural carina: NI = 1.80—2; mesopleurum basally punctate to rugosopunctate; fore wing with proximal and distal sclerites.

Distribution: India: Uttar Pradesh, Arunachal Pradesh; China; Japan; Korea; Taiwan.

KEY TO IMDIAN SPECIES OF *DICAMPTUS* SZEPLIGETI

1. Fore wing with CI = 0.15—0.20; ICI = 0.20—0.30; *Cu-a* slightly proximal or opposite to *Rs & M*; *Rs* + 2*r* elbowed; scutellum apically with longitudinal carinae; outer surface of fore tibia with few scattered spines; *1m-cu* evenly curved 2

— Fore wing with CI = 0.65—1.20; ICI = 0.60—0.90; *Cu-a* always proximal to *Rs & M*; *Rs* + 2*r* centrally sinuous; scutellum apically striate to punctate; outer surface of fore tibia with numerous long downcurved spines; *1m-cu* sinuate 3

2. Clypeus slightly convex; outer mandibular surface with tuft of elongate pubescence, teeth unequal; scutellum with 3 longitudinal apical carinae;

fenestra with a single sclerite; *M* between *3m-cu* and *3rm* straight; NI = 2.25—2.75, propodeum with a distinct carina joining rim of the spiracle to pleural carina.....*indicus* Nikam

— Clypeus somewhat nose like; outer mandibular surface with scatter pubescence, teeth equal; scutellum with 2 longitudinal apical carinae; fenestra with 2 sclerites; *M* between *2m-cu* and *3rm* curved; NI = 2; propodeum without a distinct carina joining rim of the spiracle to pleural carina..... .....*bicaritus*, sp. nov.

3. Clypeofacial suture very distinct: AI = 0.75—0.90; 1st abscissa of Cu_{1a} 1.60—1.80 times as long as 1st abscissa of Cu_1; *3rm* 0.60—0.70 the length of *2m-cu*; distal abscissa of *Rs* in hind wing with a basal stub, rest weakly pigmented, with 8 hamuli on R_1..... .....*lambai*, sp. nov.

— Clypeofacial suture indistinct; AI = 1.10—1.55; 1st abscissa of Cu_{1a} 1.10—1.50 times as long as 1st abscissa of Cu_1; *3rm* 0.75—0.90 the length of *2m—cu*; distal abscissa of *Rs* in hind wing pigmented throughout, with 9—12 hamuli of R_1..... 4

4. Mandible with short deep groove bearing long pubescence; face evenly, strongly convex; occipital width 1.75—1.95 times the maximum diameter of posterior ocellus; propodeum with a distinct carina joining rim of the spiracle to pleural carina; *Rs* 1.70—1.85 times as long as *Rs* + *2r*; fenestra with a single sclerite; NI = 3—3.50..... *reticulatus* (Cameron)

— Mandible without a groove, with sparse pubescence; face moderately convex; occipital width 1.50—1.70 times the maximum diameter of posterior ocellus; propodeum without a distinct carina; *Rs* 2—2.25 times as long as *Rs* + *2r*; fenestra with 2 sclerites; NI = 1.80—2..... *nigropictus* (Matsumura)

Acknowledgements: The authors are grateful to Prof. R. Nagabhushanam, Head, Department of Zoology, Marathwada University, Aurangabad for providing laboratory facilities and to CSIR, New Delhi for award of Junior Research Fellowship to the Junior author. Thanks are due to Ian Gauld, Commonwealth Institute of Entomology, London for confirming the identifications and the Director, Zoological Survey of India, Calcutta for sparing the material.

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Entomon, 1984, *Vol.* 9, *No.* 2, *pp.* 115 *to* 120

KARYOTYPES OF FOUR SPECIES OF HIMALAYAN BLACK FLIES (DIPTERA : SIMULIIDAE)

S. K. DEY & T. W. FUMAFARTOSOK

Cytogenetics Laboratory, P. G. Department of Zoology, Darjeeling Govt. College Darjeeling, W. Bengal, India - 734 101

(*Received 26 December 1983*)

Mitotic metaphase chromosomes from larval ganglions have been studied in four species of black flies: *Simulium (Simulium) dentatum, Simulium (Simulium) ramosum, Simulium (Eusimulium) aureohirtum* and *Simulium (Eusimulium) purii*. The diploid chromosome number is 2n = 6 in all the four species. In *S. (E.) purii* three pairs of homologous chromosomes remain somatically paired while in the other three species no such pairing is observed. Karyotypes consist of one pair of long and two two pairs of shorter chromosomes which are distinguishable from each other by total lengths and arm ratios. The chromosomes of *S. (S.) dentatum* are longer than those of the others. Each species differs from the other with respect to the absolute length of chromosomes.

(Key words: karyotypes, chromosomes, blackflies)

INTRODUCTION

The Simuliidae are among the most important insect pests and are responsible for the transmission of human and non-human onchocerciasis and avian trypansomiasis in different parts of the world (FELLIS, 1964). In India there are several records of their biting and resultant inconvenience (LEWISS, 1974; DATTA & DAS GUPTA, 1975) but the direct evidence of disease transmission is not yet established. Some amount of work has been carried out on the cytology of black flies from Canada, Australia and U S S R by several workers (ROTHFELS & DUNBAR, 1953; ROTHFELS, 1956, 1979; DUNBAR, 1959; BASRUR, 1959; CHUBAREVA & SHCHERBAKOV, 1963; BEDO, 1977; PROCUNIER, 1982; PETROVA, 1972, 1973) because of their exceptionally large and workable salivary gland chromosomes. Moreover, ROTHFELS (1956) showed that differences in chromosome pattern allowed the recognition of biologically distinct sibling species among larvae that were considered isomorphic.

Though sixteen species of black flies have been reported by DATTA (1973, 1974 a, b) and DATTA & PAL (1975) from Darjeeling and adjoining hill areas, nothing is known about their chromosomes. During the last year we have begun a cytological survey of the black flies of Darjeeling and adjoining hill areas and in the present communication, we have reported metaphase karyotypes of four species of black flies.

MATERIALS AND METHODS

The larvae of four locally available species of black flies, *Simulium (Simulium) dentatum* Purii, *Simulium (Simulium) ramosum* Purii, *Simulium (Eusimulium) aureohirtum* Brunetti and *Simulium (Eusimulium) purii* Datta were collected from slow to moderately flowing streams in and around Darjeeling hill areas.

The ganglion chromosome preparation was made from final or penultimate instar larvae

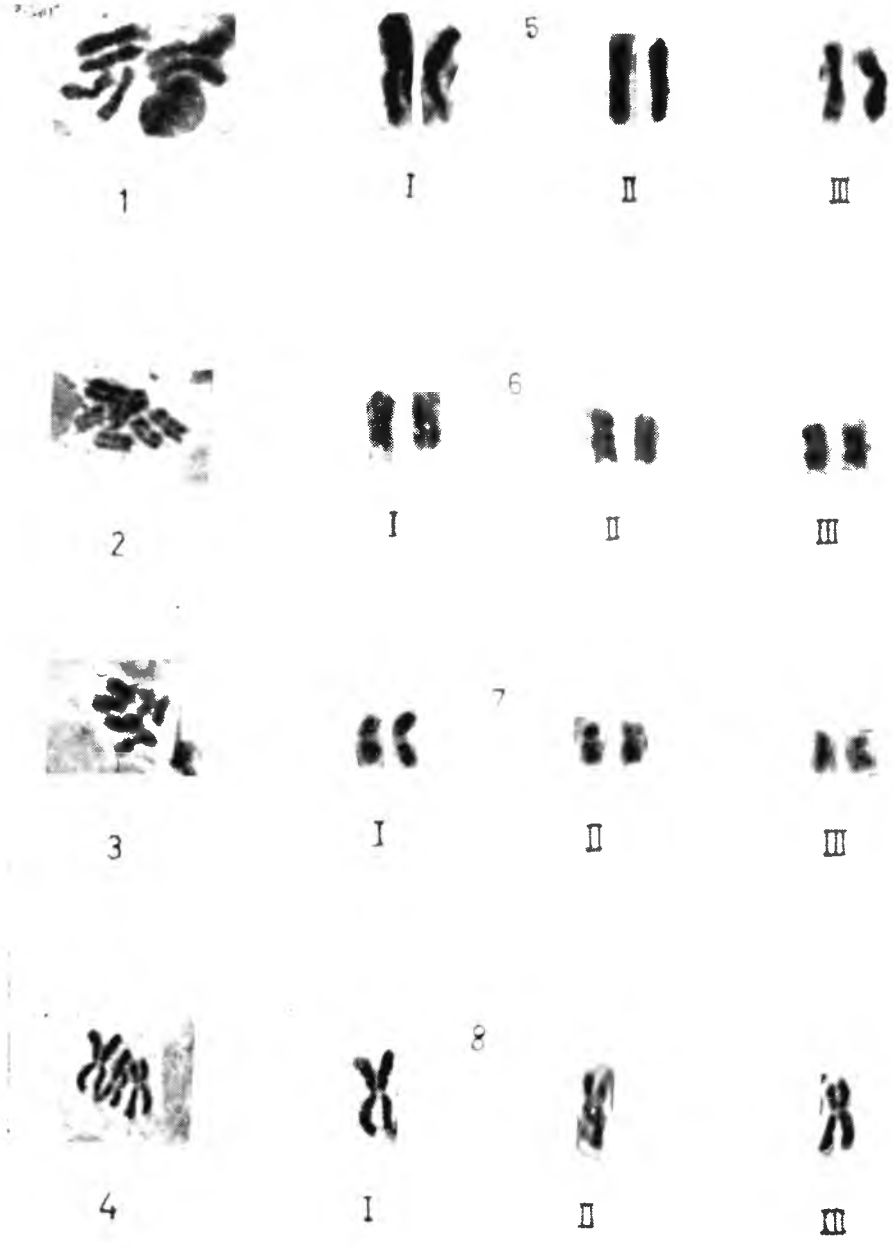
pretreated with 0.2% colchicine solution for 2–3 hr. The ganglions were stained in 2% lacto-acetic orcein solution for 15 minutes and temporary squash preparation was made. All observation and photography were perfor. med on fresh temporary preparations. Chromosomes were arranged and numbered in descending order of length using Roman numerals (I, II, III) in order to construct the karyotype. The chromosomal lengths were measured from five well spread metaphase plates and idiograms of four species were prepared (Figs. 9—12). For the study of chromosome morphology, the terminology used by LEVAN *et al.* (1964) was followed.

RESULTS AND DISCUSSION

Simulium (Simulium) dentatum Puri: The diploid chromosome number in this species is 2n = 6 (Figs. 1 & 5). Among them, one pair is long and two pairs are shorter chromosomes (Fig. 5). All the chromosomes are metacentric in nature and the homologous pairs usually lie side by side in metaphase plates (Fig. 1). The length of the chromosome ranges from 5.58 μm in the shortest chromosome to 7.80 μm in the longest chromosome. The total length of the chromosomes (n) at metaphase is 19.83 μm. Ist, IInd and IIIrd chromosomes are distinguishable from each other by total lengths and arm ratios (Table 1).

TABLE 1. Chromosome measurements of four species of Himalayan black flies. Mean of five plates.

Name of species	Chromosome No.	Mean length ± S E (μm)		Total length	Arm ratio	Centromeric index	Relative % length	Nature of Chromosomes
		Short arm	long arm	(s + l)	$r = \frac{l}{s}$	(i)		
S. (S.) dentatum	I	3.40 ±0.14	4.40 ±0.17	7.80	1.29	43.58	39.33	m
	II	2.59 ±0.17	3.86 ±0.34	6.45	1.49	40.15	32.52	m
	III	2.63 ±0.48	2.95 ±0.34	5.58	1.12	47.13	28.13	m
S. (S.) ramosum	I	1.75 ±0.09	2.22 ±0.10	3.97	1.26	44.00	41.70	m
	II	1.40 ±0.72	1.77 ±0.43	3.17	1.26	44.16	33.39	m
	III	0.97 ±0.10	1.41 ±0.92	2.38	1.45	40.75	25.00	m
S. (E.) aureohirtum	I	1.74 ±0.09	2.18 ±0.10	3.92	1.25	44.38	42.06	m
	II	0.99 ±0.12	2.03 ±0.27	3.02	2.05	32.75	32.40	m
	III	0.98 ±0.09	1.40 ±0.07	2.38	1.42	41.17	25.53	m
S. (E.) purii	I	2.84 ±0.09	3.27 ±0.09	6.11	1.15	46.48	38.86	m
	II	1.90 ±0.13	3.13 ±0.03	5.03	1.60	37.77	31.99	m
	III	1.86 ±0.10	2.72 ±0.08	4.58	1.50	40.61	29.13	m



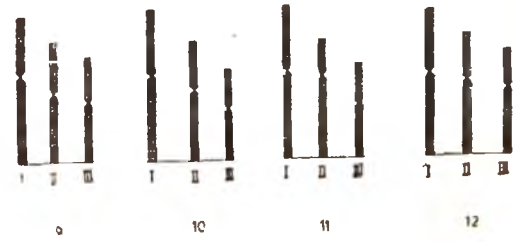
Figs. 1–4 and Figs. 5–8. Mitotic metaphase plates and karyotypes of *S. (S.) dentatum*, *S. (S.) ramosum*, *S. (E.) aureohirtum* and *S. (E.) purii* respectively, × 1500.

Simulium (Simulium) ramosum Purii: This species has also revealed 2n = 6 chromosomes (Figs. 2 & 6). Karyotype consists of one pair long and two pairs shorter chromosomes (Fig. 6). All the chromosomes are metacentric and arranged hapazardly at metaphase (Fig. 2). The cromosome length ranges from 2.38 μm to 3.97 μm. The total length of chromosomes is 9. 52 μm. Arm ratios are found to be the same in chromosome I and II, however, they differ in total length (Table 1).

Simulium (Eusimulium) aureohirtum Brunetti: The chromosome nuber of 2n = 6 is observed in this species (Figs. 3 & 7). The karyatype shows the presence of one pair long and two pairs shorter chromosomes (Fig. 7). Of these, Ist and IIIrd pairs are metacentric while the IInd pair is submetacentric. The arrangement of chromosomes at metaphase plates are similar to that of *S. (E.) dentatum*. The length of the chromosome ranges from 2.38 μm to 3.92 μm. The total chromosome length (n) is 9.32 μm. Individual chromosomes are distinguishable from each other by total lengths and arm ratios (Table 1).

Simulium (Eusimulium) purii Datta: The deploid chromosome number in this species is also 2n = 6 (Figs. 4 & 8). However, three pairs of metacentric chromosomes remain somatically paired in the mitotic metaphase plates (Fig. 4). Karyotype consists of one pair long and two pairs shorter chromosomes (Fig. 8). The length of the chromosome ranges from 4. 58 μm to 6.11 μm. The total chromosome length (n) is 15. 72 μm. Individual chromosomes are recognisable by their total lengths and arm ratios (Table 1). Moreover, karyomorphological features of this species are similar with those of *Simulium vittatum* (ROTHFELS, 1956).

According to ROTHFELS (1979), in the Simulidae mitotic karyotypes are remarkably conservative and the basic chromosome complement of this family as represented in all genera studied consist of n = 3 chromosomes with median or sub-median centromeres. However, deviation from this basic pattern has been reported in *Eusimulium aureum* (DUNBAR, 1959) and in *Cnephia lapponica* (PETROVA, 1972; PROCUNIER, 1982) where the basic chromosome complements are reduced from n = 3 to n = 2 metacentrics as a result of fusion of chromosomes II & III.



Figs. 9—12. Idiograms of *S. (S.) dentatum*, *S. (S.) ramosum*, *S. (E.) aureohirtum* and *S. (E.) purii* respectively (Based on relative percentage length of chromosomes).

All the four species of *Simulium* investigated here also have a basic chromosome number of n = 3. However, each species differs from the other with respect to the absolute length of the chromosomes. Thus, the cromosomes of *S. (S.) dentatum* are longer than those of any other species investigated here. And the chromosomes of *S. (S.) ramosum* and *S. (E.) aureohirtum* are placed in between *S. (S.) dentatum* and *S. (E.) purii*. Though each species shows its individuality in karyomorphological features, yet the natural relationship among them is supported by the chromosome number.

Acknowledgements: The authors are grateful to Dr. M. DATTA, Z. S. I., Calcutta for suggestions encouragement and helping in identification.

Thanks are due to Dr. B. Das Gupta, Principal, Darjeeling Govt. College for encouragement and Dr. D. Chakroborty, Head, Dept. of Zoology for providing laboratory facilities. The second author is thanklul to C S I R for the award of Junior Research Fellowship.

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Entomon, 1984, *Vol.* 9, *No.* 2, *pp.* 121 *to* 126

EFFECTS OF SUBLETHAL DOSES OF DIFLUBENZURON ON ENERGY BUDGET OF *ERGOLIS MERIONE* (LEPIDOPTERA : NYMPHALIDAE)

S. CHOCKALINGAM & M. KRISHNAN

Zoological Research Laboratory, Thiagarajar College, Madurai, India 625 009

(*Received 26 December 1983*)

Toxicity and the effect of orally administered sublethal doses of diflubenzuron on the energy budget of the fifth instar larvae of *Ergolis merione* have been studied. The LD_{50} value of diflubenzuron was 52.56, 27.76, 13.60 and 8.76 μg/larve for *E. merione* treated for 24, 48, 72 and 96 hr respectively. The rate of consumption was reduced by 33.34%, assimilation by 21.82% and conversion by 65.64% in the highest sublethal dose consumed larva over that of control larva. Diflubenzuron administered along with food not only reduced the growth of the larvae but also affected the growth and emergence of the adult of *E. merione* with morphological deformities.

(*Key words:* insecticde, food utilization, *Ergolis merione*, diflubenzuron)

INTRODUCTION

In the recent past the phenyl urea compounds have aroused the interest of biologists because of their novel mode of action, their specificity to arthropods and their lower ecological magnification (SUNDARAMOORTHY & SANTHANAKRISHNAN, 1979). These compounds inhibited deposition of chitin in the cuticle, impeded ecdysis, reduced adult growth and introduced morphological deformities in insects (GROSSCURT & ANDERSON, 1980; KRAURER & MCGREGOR, 1980; SAXENA & VINOD KUMAR, 1981 a, b; CHOCKALINGAM *et al.*, 1982). The growth and metamorphosis of the insects have been reported to be arrested by the topical application of phenyl urea compounds (HOLST, 1975; RATNAKARAN & SMITH, 1975; ACHER & NEMRY, 1976). Whether the oral administration of phenyl urea compounds influences the food utilization and growth in lepidopteran insects remains to be studied in detail. A reduction in the food intake was reported in larva of *Spodoptera litura* fed diflubenzuron treated leaves (SUNDRAMOORTHY, 1977). The aim of the present investigation is to evaluate the impact of sublethal doses of orally administered diflubenzuron on the bioenergy budget of a monophagous lepidopteran pest, *Ergolis merione*.

MATERIALS AND METHODS

The larvae of *Ergolis merione* were collected from the field in the vicinity of Madurai and reared in the laboratory on castor leaves at 30 ± 1°C. Four hours old final instar larvae were selected and used in this experiment.

Experimental design for toxicity studies

Diflubenzuron (25%, 1–C_4–chlorophenyl) –3 (2, 6–difluorobenzyl urea) obtained from Dr. A. B. BORKOVECK of USDA, USA, and M/s. Mysore Insecticides and Co., Madras was used in the present study. Solutions of different (active ingrediants) strength of diflubenzuron were prepared by dissolving it in

acetone. Four hours old final instar was introduced into separate petri-dishes (20 larvae were maintained separately for each dose) containing castor leaf bits sprayed with various doses of diflubenzuron. The percentage of larval mortality and the toxicity range of the toxicant for *E. merione* were noted at intervals of 24, 48, 72 and 96 hr. Median lethal doses (LD_{50}) were assessed for different durations of feeding schedule of 24, 48, 72 and 96 hr by plotting the percentage mortality against the different doses of diflubenzuron. From the graph, the dose at which 50% of individuals died over a period of 24, 48, 72 and 96 hr was derived by straight line graphical interpolation method (KING, 1962).

Experimental design for feeding

For food utilization experiment, 4 hr old final instar larvae of *E. merione* were maintained individually in a 250 ml plastic container. For each experiment five replicates were maintained. The desired dose of diflubenzuron (0.88, 1.70 and 3.40 µg) was applied on small pieces of weighed middle aged castor leaf with the help of a micro applicator and then the larvae were allowed to consume the treated leaf pieces entirely. Thus a known quantity of diflubenzuron was orally administered to the fifth instar larva only the first day. Subsequently the larva was allowed to feed *ad lioitum* on fresh middle aged castor leaves, free from diflubenzuron treatment. Control larvae were fed acetone treated castor leaves. Larvae, faeces and unfed food were dried to a constant weight at 90°C. All the materials were weighed to an accuracy of 0.01 mg in a monopan balance. Calorific content of food, faeces and test individuals were estimated in a Pan 1411 semi-micro bomb calorimeter following the standard procedure described in the instruction for Pan Bomb Calorimeter No. 128 and 130. Data obtained in dry weight were converted into calorie, applying the calorific values.

The scheme of energy budget followed in the present study is a slightly modified IBP formula (PETRUSEWICZ & MACFADYEN, 1970) represented as $C = P + R + (F + U)$ where 'C' represents consumption, 'P' production, R and F energy loss *via* respiration and faeces production respectively; it has been described in detail elsewhere (KRISHNAN & CHOCKALINGAM, 1981).

RESULTS

Toxicity

An extention of the feeding schedule of the larvae to diflubenzuron increased the mortality. The regression for the relation between the doses of diflubenzuron and mortality of the larvae was calculated for different hours of feeding schedule. The regressions obtained were:

$Y = -18.91 + 1.311 \times$ for 24 hr
$Y = 9.42 + 1.462 \times$ for 48 hr
$Y = 27.58 + 1.648 \times$ for 72 hr and
$Y = 22.68 + 3.120 \times$ for 96 hr.

Survival

LD_{50} value was determined for *E. merione* treated to 24, 48, 72 and 96 hr series. It was higher for the larvae treated for shorter duration than for those treated for longer duration for instance LD_{50} decreased from 52.56 µg/larva for 33 hr to 27.76, 13.60, 8.76 µg/larva in the 49, 72 and 96 hr series respectively (Fig. 1).

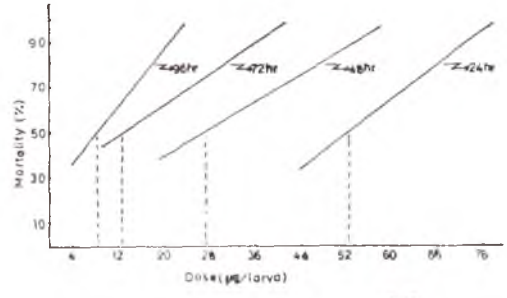

Fig. 1. Dotted lines indicate the LD_{50} value of diflubenzuron for the fifth instar larva of *E. merione* exposed to different hours.

Food utilization

The larva treated with very low sublethal dose of 0.88 µg (10% of the

TABLE 1. Effect of orally administered sublethal doses of diflubenzuron on the energy budget of the fifth instar larvae of *E. meriane*. All values are expressed in gcal/individual. $\bar{x} \pm$ S D.

doses of diflubenzuron consumed (μg/larva)	Consumption	Faeces	Assimilation	Production	Metabolization
Control (0.00)	895.09 ± 40.32	415.60 ±16.38	479.49 ±30.04	143.24 ±17.10	336.25 ±15.92
0.88	1206.34 ±110 24	467.77 ±14.24	738.57 ±41.13	134.05 ±10.53	604.52 ±16.27
1.70	810.54 ± 60.53	290.22 ±11.05	520.32 ±35.12	83.94 ± 8.49	436.38 ±17.56
3.40	640.32 ± 51.45	224.38 ±13.63	415.94 ±32.83	59.24 ± 7.26	356.70 ±15.67

LD_{50} value) consumed (1206.34 gcal) and assimilated (738.57 gcal) more than that of control larva (895.09 and 479.49 gcal) (Table 1, Fig. 2). Rate of consumption, assimilation and conversion of food in the larva decreased when diflubenzuron doses applied on the leaves increased. Feeding rate, which was 1884.40 gcal/g live wt/day in the control larva decreased to 1255.26 gcal/g live wt/day in those consumed the highest sublethal dose (3.40 μg). Likewise, the rate of conversion also decreased from 303.13 gcal/g live wt/day in control larva to 220.20, 149.53 and 94.26 gcal/g live wt/day when the larva consumed 0.88, 1.70 and 3.40 μg of diflubenzuron along with food (Table 2, and Fig. 3).

Assimilation efficiency was directly related to the dose of diflubenzuron consumed by the larvae ($r = 0.818$, $P < 0.025$). However, the conversion efficiency progressively decreased from 30.03% in the control to 17.96, 16.27, 14.59% in the larva fed on 3.40, 1.70 and 0.88 μg of diflubenzuron treated leaves (Table 2).

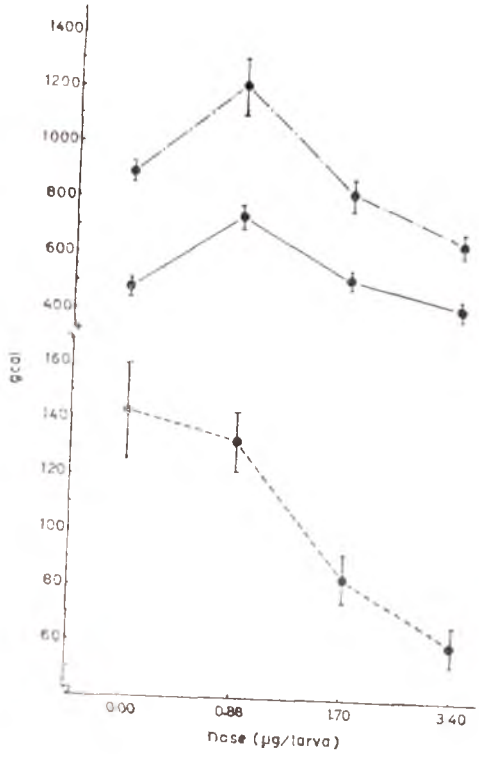

Fig. 2 Effect of sub-lethal doses of diflubenzuron on the food energy consumption (upper) assimilation (middle) and conversion (lower) in the fifth instar larva of *E. meriane*.

TABLE 2. Effect of orally administered sublethal doses of diflubenzuron on the energy budget of the fifth instar larva of *E. merione*. Rates are in gcal/g live wt/day and efficiencies in percentage. $\bar{x} \pm$ SD.

Doses of diflubenzuron consumed (μg/larva)	Consumption rate	Assimilation rate	Production rate	Assimilation efficiency	Conversion efficiency
Control (0.00)	1884.40 ± 116.62	1009.45 ± 133.85	303.13 ± 30.39	53.58 ± 1.59	30.03 ± 4.32
0.88	2002 22 ± 128.81	1225.84 ± 121.69	220.20 ± 27.06	61.22 ± 2.99	17.96 ± 3.26
1.70	1472.69 ± 96.82	918.25 ± 137.97	149.53 ± 17.55	62.35 ± 0.82	16.27 ± 1.57
3.40	1255.26 ± 103.56	795.73 ± 109.45	94.26 ± 14.84	63.44 ± 1.83	14.59 ± 1.64

	Feeding Rate	Production Rate	Conversion efficiency
0.00 Vs 0.88	t = 1.52 NS	t = 4.43*	t = 4.12 NS
0.00 Vs 1.70	t = 6.08*	t = 9.79*	t = 6.71*
0.00 Vs 3.40	t = 9.02*	t = 13.81*	t = 7.50*

NS – Not Significant
* – Significant ($P < 0.01$).

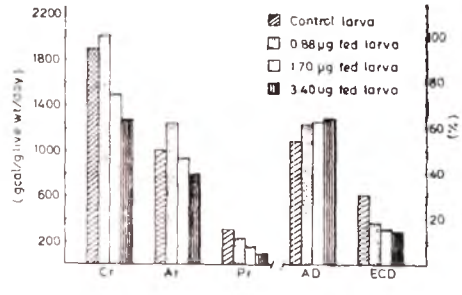

Fig. 3. Effect of sublethal doses of diflubenzuron on the rate of food energy consumtion (Cr) Assimilation (Ar) Conversion (Pr) and efficiencies of Assimilation (AD) and conversion (ECD) in the fifth instar larva oy *E. merione*.

DISCUSSION

The LD_{50} value of ingested diflubenzuron for *E. merione* at 48 hr treatment was two times lower (27.76 μg/larva) when compared to that of the same compound (43.90 μg/larva) for another lepidopteran pest, *Pseudoplusia iucludens* (REED & BASS, 1980) indicating that diflubenzuron's activity is species specific. ACHER & NEMRY (1976) have found that diflubenzuron ingested together with the wheat bran baits has differntial activity in larva of polyphagous species such as *Spodoptera littoralis* and *Agrotis ypsilon*. It was highly toxic to the larva of *S. littoralis* even at the lower doses while such doses were ineffective to *A. ypsilon*. The monophagous (DAVID & KUMARASWAMI, 1978) *E. merione* seems to be more susceptible to the action of ingested diflubenzuron even at the lower dose than the polyphagous species such as *P. includens* and *S. littoralis*. It may be due to the polyphagous species of Lepidoptera,

feed on taxonomically diverse host plants, have developed broader range of defensive compounds (CATES, 1981) which are likely to be capable of detoxifying the consumed insecticides and thus reduce the efficiency of the action of ingested insecticides in polyphagous species.

A slight increase in the consumption and assimilation of food was noticed in the low sublethal dose fed larvae of *E. merione*. Toxins are usually neutralized by the herbivorous insects. The effect of such toxins, which cannot be fully neutralized or can only be partially neutralized was found to be compensated by a considerable increase in food energy consumption and assimilation (KASTING & MCGINNIS, 1963; MATHAVAN & BASKARAN, 1975). The reduction in the weight gain by the larvae of *E. merione* consumed highest sublethal doses of diflubenzuron suggest that the chemical might have impaired the physiological systems other than the deposition of chitin.

The increase in assimilation efficiency (42.75% over that of control) concomitant with the reduction in the rate of feeding in the larvae of *E. merione* fed on different doses of diflubenzuron over that in control larvae may be due to the higher food retention time in the gut and also due to slowing down the peristaltic movement of the gut caused by the action of insecticides (PRICE, 1975; MATTSON, 1980). The reduction in conversion efficiency and growth in the diflubenzuron fed larva of *E. merione* recalls the result that herbivore insects are committing more of its energy resources for the tolerance of insecticide action and detoxification than for the body growth (KRIEGER *et al.*, 1971; BROWER & GLAZIER, 1975).

Diflubenzuron administered along with food not only reduced the growth of final instar larva but also affected the growth and emergence of adult moth in *E. merione*. The adult emerged from the treated larva varied from that of control insects showing morphological abnormalities such as reduced body size, crippled and folded wings and curved appendages. It may be due to the blockage of deposition of chitin in the endocuticle as has been reported in the larva of *Pieris brassicae* (POST & VINCENT, 1973) and housefly (ISHAAYA & CASIDA, 1974).

The fact that growth in diflubenzuron treated final instar larva of *E. merione* in the present study and in soybean looper, *P. includens* (REED & BASS, 1979) was less than that in the untreated larva over equal units of time makes the use of diflubenzuron more attractive in the lepidopteran pest management system.

Acknowledgements: We wish to express our thanks to Dr. (Mrs.) RADHA THIAGARAJAN, Mr. T. KANNAN, Dr. S. P. ANNAMALAI, and Dr. S. KANNAN of our College for providing facilities and encouragement. We thank Dr. T. J. PANDIAN, School of Biological Sciences, Madurai Kamaraj University for permitting to use the semi microbomb calorimeter and Dr. S. C. SAXENA, for valuable suggestions. Supply of diflubenzuron by Dr. A. B. BORKOVEC, and M/s. Mysore Insecticidea and Co., Madras and financial assistance from Madurai Kamaraj University, U G C and C S I R, New Delhi are gratefully acknowledged.

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Entomon, 1984, *Vol.* 9, *No.* 2, *pp.* 127 *to* 133

STUDIES ON THE LIFE HISTORY OF INDIAN DRAGONFLY *ANAX IMMACULIFRONS* (RAMBUR) (AESHNIDAE : ODONATA)

ARUNKUMAR

Northern Regional Station, Zoological Survey of India, Dehra Dun, India 248 001

(Received 9 August 1983)

Anax immaculifrons has been reared from egg to adult. The principal changes in external morphology during the development of larval instar and the characters which are helpful in distinguishing various instars have been discribed.

(Key words: life hisrory, Indian dragonfly, *Anax immaculifrons)*

INTRODUCTION

Consequent to my studies on the larval stages and the life history of Indian dragonflies, present is the tenth paper in the series. In this paper findings are presented of the study on the larval stages of *Anax immaculifrons* (Rambur). Descriptien of the last instar larva of this species and a key to the known larvae of 5 Indian species of genus *Anax* have been provided earlier (Sangal and Kumar, 1970; Kumar, 1973).

Corbet (1955, 1957), in his classic work, has made extensive study on the larval stages, life history and ecology of the emperor dargonfly, *Anax imperator* (Leach) from Britain, which helped him to formulate, now widely accepted ecological classification of the Brittish Odonata. In addition, the life history of the large and beautiful dragonflies of this genus have been studied in detail in temparate region by a number of Odonatologists, viz., Tillyard (1916), Munschberg (1932), Calvert (1934) and Robert (1939). Though 7 species of this genus are recorded from INDIA (Kumar, 1973), no attempt has so far been made to study the larval development of any species. The purpose of the present paper is to fulfil this requirement.

MATERIAL AND METHODS

On 22.iv.1976 a few eggs were collected from a submerged leaf, in which a female was observed ovipositing, at a small shady hill stream in Rajpur, Dehra Dun. The leaf was kept submerged in water in a glass trough in the laboratory. Hatching occurred on 7.v.1976 and 10.v.1976 (two larvae). These two larvae (1 & 2) were kept separately. Larva 1 died in 12th instar while larva 2 emerged into a male imago on 1.ix.1976 after passing through 13 instars, thus having taken 132 days from date of oviposition to emergence. The dates of ecdyses during the growth of these two larvae are given in Table 1.

The morphological data regarding the larval development is derived from these two larvae. Though Corbet (1955) has recorded that the number of instars may vary, useful characters for determination of the instar have been given. Lerval measurements are provided as indication of approximate size. Larval length was measured from tip of head to tip of anal appendages.

The egg: (Fig. 1) is elliptical in shape with a blade like projection at

TABLE 1. Breeding record (1976) of two reared larvae of *Anax immaculifrons* (Rambur)

Eggs collected on 22.4.1976
Eggs hatched on 7.5.1976

Duration of egg stage 15 days

Both the eggs which gave rise to larvae recorded in the table, hatched on 7.5.1976.

	Duration (days)		Average (maximum) temperature during the period
	Larva 1	Larva 2	
2nd	3	4	
3rd	7	6	
4th	4	5	
5th	5	6	
6th	6	8	
7th	5	5	
8th	7	8	24°—37°C
9th	9	10	
10th	11	16	
11th	11	9	
12th	Died on 14.7.1976	14	
13th		26	
Eemergence		1.9.1976	
Total number of days from oviposition to emergence		132	

the anterior pole: presence of this projection has been recorded in other species of *Anax* as well, viz., *A. imperator* Leach (Robert, 1939: Corbett, 1955), *A. junius* (Drury) (Needhham and Betten, 1901) and *A. papuensis* Burm. (Tillyard, 1916). Robert (vide Corbet, 1955) while discussing the possible function of this cone has suggested that it possibly prevents the imprisonment of the egg by the overgrowth of the surrounding plant tissue or provides a safe passage for release of larva at the time of hatching.

Larval development: The prolarva has a very brief duration. The external morphological changes from 2nd to 13th instar are described below. Summary of principal larval characters is givan in Table 2.

Head: During the larval development of *A. immaculifrons* the shape of head and eyes changes distinctly from instar 2 to instar 6, upto which time it attains the characterstic shape of the genus *Anax*. In instar 2 the head is broadly triangular with antero-laterally placed eyes. Their shape changes and they extend posteriorly as the development proceeds. Following this the head became roundish and attains the typical *Anax* shape by instar 7. Corbet (1955) recorded that in instar 2 the larva of *A. imperator* to a large extent resembles that of *Aeshna* because of peculiar head outline.

Table 2. Summary of larval development of *Anax immaculifrons* (Rambur).

Instar	1	2	3	4	5	6	7	8	9	10	11	12	13
Antennal segments		3	3	4	4	5	5	5	6	7	7	7	7
Tarsal segments		1	1	2	2	2	2	3	3	3	3	3	3
Abdominal segments covered with wing buds		—	—	—	—	—	—	$\frac{1}{2}$	$\frac{3}{4}$	1	$1\frac{1}{2}$	$2\frac{1}{2}$	$4\frac{1}{2}$
Anal cerci		—	—	—	—	+	+	+	+	+	+	+	+
Gonopophyses		—	—	—	—	+	+	+	+	+	+	+	+
Head width (mm)		0.88	1.20	1.65	1.80	2.25	3.42	4.40	5.57	6.80	8.10	9.0	10.10
Body length (mm)		2.2	3.5	4.2	5.7	6.5	8.0	9.2	15.4	26.0	38.1	45.2	54.2

(—) absent; (+) present

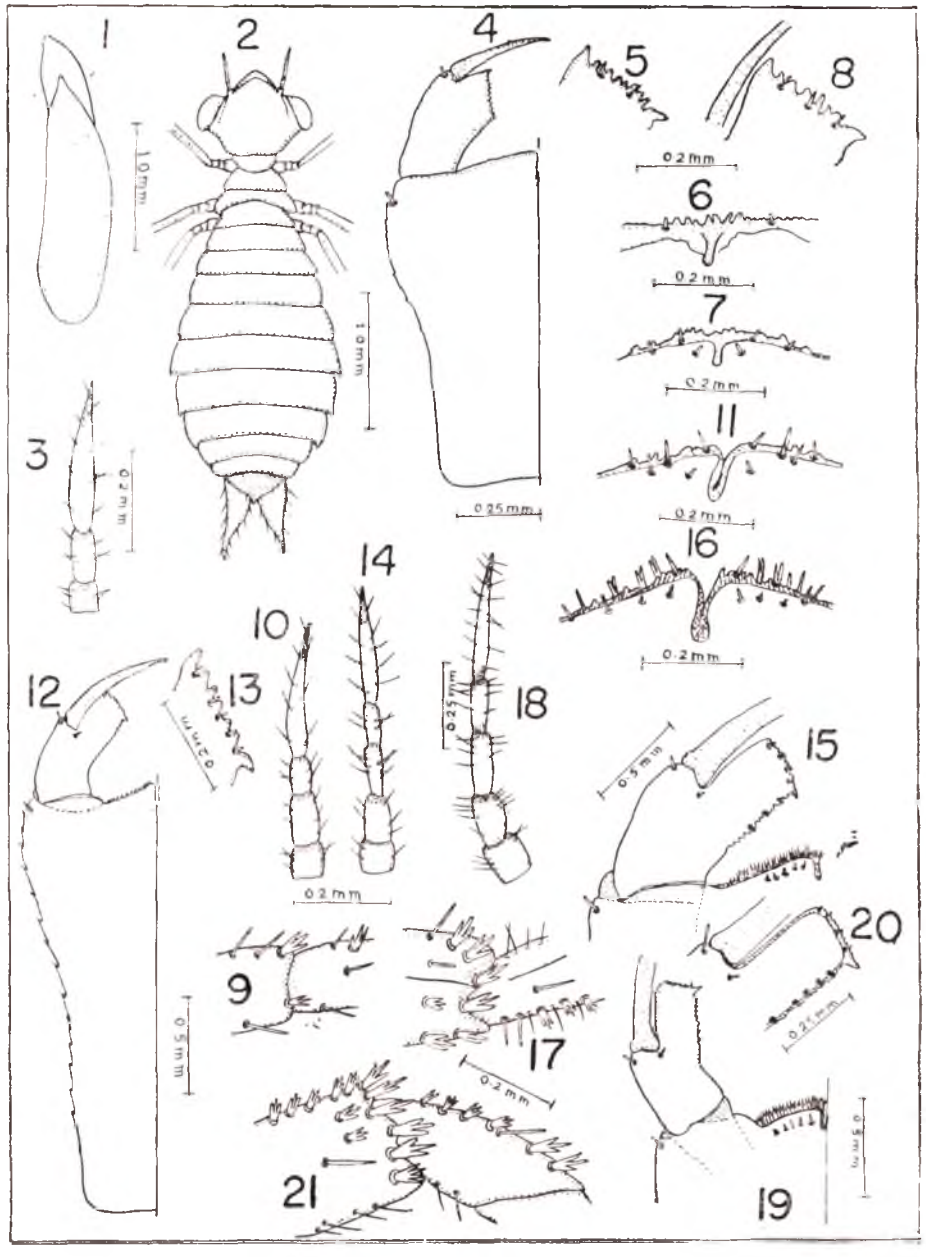
In each instar the size and width of head increase considerably. Corbet (1955) recorded it to be between 10 to 30 percent in case of *A. imperator*.

Antennae: In instar 2 the antennae are three-segmented (Fig. 3). They became four-segmented in instar 4 (Fig. 10), five-segmented in instar 6 (Fig. 14) and posses the final seven-segments in instar 10. From three to six-segmented condition the additional segments are added by the division of proximal flagellar segment; the seventh segment is added by the division of the apical segment of flagellum. It corresponds with the antennal development of *A. junius* (Calvert, 1934) and *A. imperator* (Corbet, 1955).

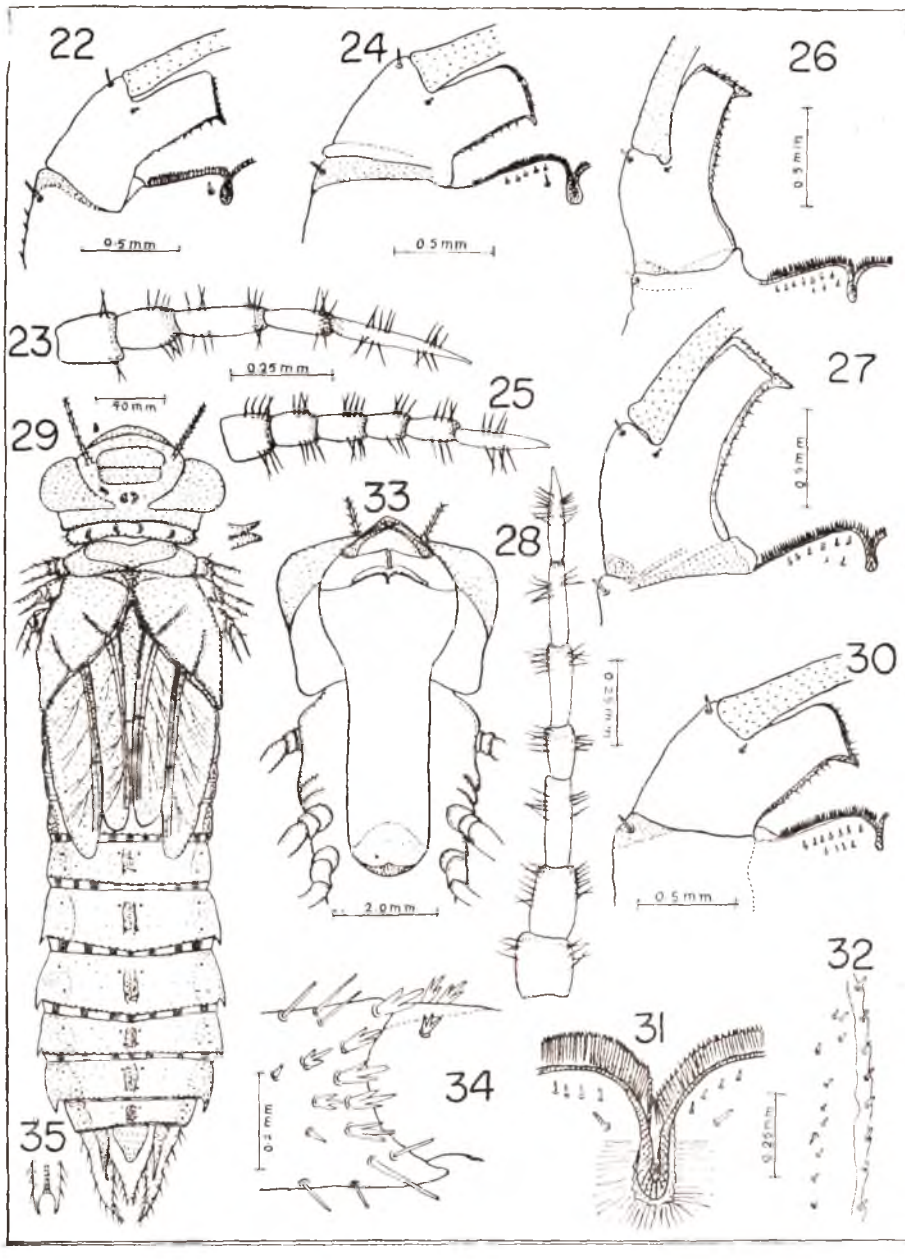
Labium: From instar 2 to 13 the general shape of the labium does not change much and remain characterstically that of the genus *Anax* (Figs. 4 to 30). Major changes occur only in the shape and structure of the distal margin of median lobe of prementum and inner margins of palpus.

Median lobe: Its prominent feature is the median cleft which becomes distinct from instar 2 onwards. The distal margin is formed into five spine-like crenations on either side of the cleft, a seta is present on either side with the outermost spine in instar 2 (Fig. 6). From instar 3 onwards the number of setae increases on either side of the cleft (Figs. 7, 11 and 16), so that each spine has a seta on its dorsal side. In addition, from instar 6 onwards a series of piliform setae arise from the ventral side of the distal margin of median lobe (Figs. 15 and 16). A number of these appear to arise in pairs, with larval development number of these setae increases rapidly on distal margin (Figs. 22, 24, 26, 27 and 30) and by 10th instar they become too numerous to count and seem to provide the main setal armature of the distal margin.

Palpus: In instar 2 the distal margin of palpus (Figs. 4 and 5) is formed into deep dentations, 2 small setae are present on these, another seta is added to it in



Figs. 1. egg; Figs. (2–6). 2nd Instar larva: 2. Larva; 3. antenna; 4. labium; 5. distal margin palpus (E. V.); 6. distal margin median lobe (E. V.); (7—9). 3rd instar larva: 7. distal margin median lobe (E. V.): 8. distal margin palpus (E. V.); 9. tibial comb; (10 and 11). 4th Instar larvae; 10. antenna; 11. distal margin median lobe (E. V.); (12 and 13). 5th Instar larva; 12. labium; 13, distal margin palpus (E. V.); (14–17). 6th Instar larvae; 14 antenna; 15. labium; 16. distal margin median lobe (E. V.); 17. tibial comb; (18–21). 7th Instar larva; 18. antenna; 19. labium; 20. distal margin palpus (E. V.) 21. tibial comb;



Figs. 22 nd 23. 8th Instar larva; 22. labium; 23. antenna; (24 and 25). 9th Instar larva; 24. labium; 25. antenna; 26. labium (10th Instar larva); (27 and 28). 11th Instar larva; 27. labium; 28. antanna; (29–35). 13th Instar larva; 29. larva; 30. labium 31. distal margin median lobe (E. V.); 32. lateral margin palpus (E. V.) 33. anoral view of the labium; 34. tibial comb; 35. distal margin epiproct (E. V.).

instar 3 (Fig. 8). Corbet (1955) has recorded similar setae in the palpus of *A. imperator* and has suggested that these may be homologous to those observed in *Sympetrum striolatum* (Fam. Libellulidae). These setae further increase in number with the larval development. In addition setae also develop on the inner margin of palpus.

A single robust seta is present in instar 2 near the base of movable hook (Fig. 4). It is joined by second such seta towards the inner side at the base of the movable hook (Fig. 12). Pair of these are present throughout the larval development, though they become smaller as development proceeds. Presence of such paired setae has also been recorded during the larval development of *A. junius* (Calcart, 1934) and *A. imperator* (Corbet, 1955). Corbet (1955) has proposed that in all probability these seatae are homologous to the primary palpal setae (which appear in instar 2) of the family Coenagriidae and Libellulidae. It would be further informative to study the fate of these setae in family Gomphidas, where again well developed palpal setae are not present in the larval labium.

Wing-buds: Rudimentary wing-buds in the form of pleural ridges on meso- and mata-thorax appear first in instar 6. In instar 7 they are present as small dots. They cover $\frac{1}{2}$ of first addominal segmemt in instar 8. From instar 9 to instar 13 their development is rapid. Corbet (1955) has shown that in *A. imperator* the number of addominal segments covered with wing buds provide a very useful character for differentiation at least last 3 larval instars both in field and in the laboratory. In *A. immaculiforns* the development of wing-buds as summarized in Table 2, is almost akin to Corbet's observations on *A. imperaotr*. Thus it suggests to be a useful identification character.

Anal cerci: These appear first in instar 6 and, then during larval development increase in size.

Colouration: When hatched, larvae are sienna coloured. Instar 2 to 5 the larvae are shining sienna coloured and thereafter they start becoming duller. However, this colour pattern in early instar varies considerably with the observations of Corbet (1955) on *A. imperator*. The larvae of *A. immaculifrons* are generally found in shallow pools with putrifying leaves which are almost sienna to black. It is thus possible that the young larvae resemble their background. The mature larves are dark greenish-brown and densely pigmented.

Acknowledgements: Grateful thanks are due to Dr. B. S. Lamba, Joint Director, the then Officer-in-Charge, Northern Regional Station, Zoological Survey of India, Dehra Dun and to the Director, Zoological Survey of India, Calcutta, for permission to undertake the persent work, laboratory facilities and encouragement.

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Entomon, 1984, *Vol.* 9, *No.* 2, *pp.* 135 *to* 140

STUDIES ON TWO EARWIG SPECIES COMMONLY OCCURRING IN THE SUGARCANE ECOSYSTEM

S. EASWARAMOORTHY & V. NANDAGOPAL

Sugarcane Breeding Institute, Coimbatore - 7, India

(Received 9 July 1983)

Biology and food habits of two earwig species, *Euborellia stali* (Dohrn) and *Proreus* (near) *ramamurthi* Kapoor, commonly occurring in sugarcane ecosystem were studied in the laboratory at 27 ± 1°C. Sugarcane bits served as food for nymphs and adults. The mean fecundity was 28 and 27.3 eggs in *E. stali* and *P. ramamurthi* respectively and the incubation period of eggs ranged from 5 to 8 days. There were five nymphal stadia extending upto 73.5 days in *E. stali* and 63.4 days in *P. ramamurthi*. After egg laying the adult females lived for 30 to 54 days in *E. stali* and 24 to 50 days in *P. ramamurthi*. Individuals of both the species showed distinct food preference for cane bits and they could not complete their life cycle when reared on different life stages of sugarcane shoot borer and internode borer and mealy bugs alone. *E. stali* was found to feed on borer larvae and mealy bugs, though the individuals died after a few days, while *P. ramamurthi* failed to do so. Among the two species, *E. stali* was common in pitfall trap collection and population of both the species was more during August under field conditions and it decreased progressively upto December.

(Key words: earwigs, sugarcane ecosystem)

INDRODUCTION

The predatory habits of earwigs was known as early as 1886 when Reily reported *Forficula* sp. destroying *Icerya* in USA. Since than there were a number of reports about the role played by dermapterans in the population dynamics of crop pests (BISHARA, 1934; AMAR & FARRAG, 1974; WALKER & NEWMAN, 1976; BUSHMAN *et al.*, 1977; PRICE & SHEPARD, 1978; SHEPARD *et al.*, 1978). In the beginning of this century, TERRY (1905) observed earwigs in the sugarcane fields of Hawaii and considered two of them *Euborellia annulipes* Lucas and *Chelisoches morio* (Fab.) as of considerable economic importance in the regulation of the hopper and caterpillar pests. In India two species of earwigs *Proreus simulans* Stal. and *P. melanocephalus* Dohrn were reported during 1908—1915 from the leaf sheaths and boreholes of sugarcane and paddy stubbles in North India (HEBARD, 1923). Recently from peninsular India, *E. annulipes*, *Proreus ramamurthi* Kapoor and *Labia* sp. were reported to attck sugarcane borers (RAMAMURTHI & SOLAYAPPAN, 1979, 1980). Detailed studies were made recently on the biology, feeding habits and field abundance of *E. stali* (Dohrn) and *P.* (near) *ramamurthi* which are commonly found in the sugarcane fields at this Institute to find out the role played by them in the sugarcane pest regulation.

MATERIALS AND METHODS

The earwigs collected from sugarcane fields at Coimbatore were maintained on cane pieces of 5 cm length in plastic boxes

individually at 27 ± 1°C. The cane pieces were changed once in three days. The feeding tests were conducted by providing known number of eggs, larvae of different instars of borers and mealy bugs and number killed was noted the next day and replaced with fresh individuals to maintain the number constant. In the case of feeding with sugarcane juice, cotton swabs dipped in juice was placed inside the cages and changed on alternate days. The dead earwigs were also replaced daily. For mating studies a pair of adult was allowed in a plastic box and after coitus the female was removed and reared separately. The female was allowed to guard her eggs and also the young ones upto 10 days after which the young ones were removed and maintained individually. In each experiment three replications were maintained with four to 15 days old nymphs in each replication.

A study was also conducted to find out the abundance of earwigs in cane fields by setting 10 pitfall traps at 100 feet distance. Wide mouth glass bottles (6 cm dia ×12 cm ht) were buried in soil in such a way that the upper rim of the bottle was exactly at the ground level. It was covered with an inverted 'V' shaped card board roof resting on legs of 5 cm height. In the bottles water was poured to a height of 5 cm. A little quantity of kerosene was also poured over water which acted as killing agent. The trapped insects were collected at weekly interval and sorted.

RESULTS AND DISCUSSION

Biology: Under laboratory conditions *E. stali* completed its nymphal period in 73.5 days on an average whereas *P. ramamurthi* in 64.3 days (Table 1). There were five instars in both the species which varied in body length and head capsule width. In the adults, copulation lasted for 3 to 8 minutes in *E. stali* and 4 to 6 minutes in *P. ramamurthi*. The oviposition period extended over a span of five days and the total number of eggs laid per female varied from 11 to 55 in *E. stali* and 19 to 56 in *P. ramamurthi*. The females were found to lay eggs more than once.

TABLE 1. Biology of *E. stali* and *P. ramamurthi*.

Observations	*Euborellia stali*	*Proreus ramamurthi*
Mating time (minutes)	5.5 (3—8)	5 (4—6)
Preoviposition period (days)	9.3 (6—14)	8
Number of eggs laid/female	28 (11—55)	27.25 (19—56)
Egg period (days)	5.6 (5—8)	6
Nymphal period (days)	73.5 (68—79)	64.33 (58—71)
Longevity after egg laying (days)	47.75 (36—54)	32.0 (24—50)
Total life cycle (days)	125 85 (109—141)	102.33 (88—127)

Figures in parentheses indicate the range.

The eggs were creamy white and cylindrical and found glued together to the container. The incubation period ranged from 5 to 8 days. The per cent hatching of eggs was calculated in *P. ramamurthi* which ranged from 70.37 to 100 with mean of 91.01. The mother showed both brood and post-eclosion care. After egg laying the adult females lived for 30 to 54 days in *E. stali* and 24 to 50 days in *P. ramamurthi*. The results are in corroboration with the earlier studies though the species involved are different. In *E. annulipes* KLOSTEMEYER (1942), NEISWANDER (1944) and KNABKE and CRIGARICK (1971) observed five instars. BHARADWAJ (1966) reported 83.6 days nymphal period, 0.5 to 6 minutes copulation time and 52.7 eggs/clutch in *E. annulipes*.

Food habits: Both *E. stali* and *P. ramamurthi* showed a distinct preference for sugarcane bits than for an insect diet. The mean per cent survival of the earwigs was more when reared on sugarcane bits or when sugarcane bits were provided with either internode borer *Chilo sacchariphagus indicus* K. (Table 2) larvae or mealy bugs (Fig. 1). There was species variation when sugarcane shoot bit is provided as food. In *E. stali,* 75 percent of the individuals were able to complete their life cycle on shoot bits when provided along with shoot borer *C. infuscatellus* Snell larvae whereas in *P. ramamurthi* only 16.7 percent completed their life cycle. None of the individuals in either of the species could complete their life cycle when reared on first or fourth and fifth instar larvae of shoot borer, eggs or fourth and fifth instar larvae of internode borer or mealy bugs. Similarly when fed with sugarcane juice or sugar cubes the earwigs failed to survive long.

The mean number of days of survival was more, only when the individuals

TABLE 2. Survival of *E. stali* aud *P. ramamurthi* on different diets.

Treatments	*E. stali*			*P. ramamurthi*		
	Per cent survival	Adults obtained		Per cent survival	Adults obtained	
		Male	Female		Male	Female
1. Shoot borer larva alone–first instar	0. 0	0	0	0. 0	0	0
2. Shoot borer larva alone–third and fourth instars	0. 0	0	0	0. 0	0	0
3. Shoot borer larva–third and fourth instars along with shoot bits	75. 0	6	3	16.70	2	0
4. Internode borer–egg	0. 0	0	0	0. 0	0	0
5. Internode borer alone–third and fourth instars	0. 0	0	0	0. 0	0	0
6. Internode borer larva–third and fourth instars along with cane bits	100. 0	6	6	83.33	5	5
7. Mealy bugs alone	0. 0	0	0	0. 0	0	0
8. Mealy bugs on sugarcane bits	83.33	5	5	83.33	5	5
9. Sugarcane bits alone	100. 0	7	5	91.67	5	6
10. Sugarcane juice	0. 0	0	0	*NT	—	—
11. Sugar cubs	0. 0	0	0	0. 0	0	0
12. Check	0. 0	0	0	0. 0	0	0

*NT: Not tested.

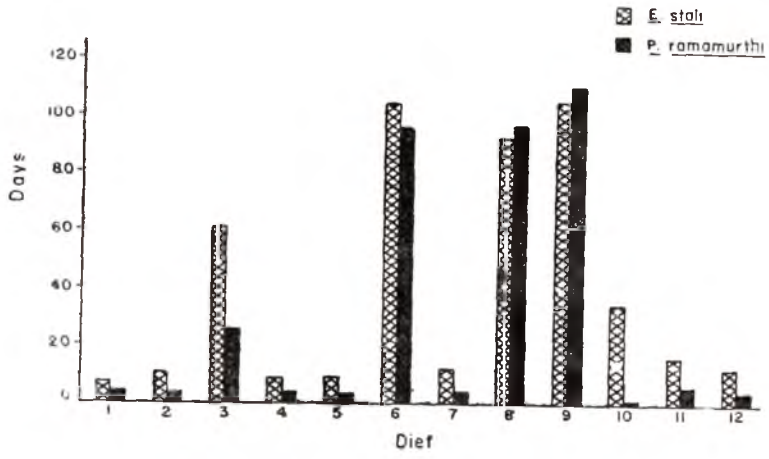

Fig. 1. Mean number of days of survival on different dieths 1. Shoot borer larvae alone, first instar; 2. Shoot borer larvae alone, third and fourth instar; 3. Shoot borer larvae, third and fourth instar along with shoot bits; 4. Internode borer eggs 5. Internode borer alone, third and fourth instar; 6. Internode; borer larvae, third and fourth instar along with cane bits; 7. Mealy bugs; 8. Mealy bugs on sugarcane bits; 9. Sugarcane bits alone; 10. Sugarcane juice; 11. Sugar cubes; 12. Check.

were reared on sugarcane bits which was provided either alone or with internode borer larvae or mealy bugs. It ranged from 93.16 to 105.83 days in *E. stali* and 96.42 to 110.33 days in *P. ramamurthi*. The survival period was considerably less when reared on shoot bits and was equal to that in check when reared on shoot borer larvae, internode borer larvae or eggs or mealy bugs alone.

E. stali was found to feed on shoot borer and internode borer larvae and mealy bugs though the earwigs died after a few days, while *P. ramamurthi* did not feed on the insects tried (Table 3). RAMAMURTHI & SOLAYAPPAN (1979, 1980) reported that *E. annulipes* and *P. ramamurthi* nymphs and adults were found to attack sugarcane borers. But it is evident from the present investigation that though only *E. stali* may attack a few larvae in its life time, it could not complete its life cycle either on borers or mealy bugs and hence not strictly predaceous. Similar results have been reported in *E. annulipes*. It was leported predaceous on the sugarcane reaf hopper *Perkinsiella saccharicida* Kiraaldy in Hawaii (TERRY, 1905), the banana root borer *Cosmopolites sordidus* German in Jamaica, on the larvae of owlet moth, *Sesamia inferens* Walker and to some extent on stored grain insects (KLOSTERMEYER, 1942). But it is a pest in Irish and sweet potatoes in storage in Mississippi, in flour mills in Kansas and in corn processing plants in Indiana, Illinois, Iowa and Missouri (GOULD, 1948) and vegetables in Ohio (NEISWANDER, 1944).

Field abundance: Among the two species studied *E. stali* was more common (Fig. 2). Both the species were more abundant during August when the study

TABLE 3. Feeding potential of *E. stali* and *P. ramamurthi*.

Treatments	*E. stali*		*P. ramamurthi*	
	Number of days observd	Average number killed/day	Number of days observed	Average number killed/day
1. Shoot borer larvae alone kept under normal light	26	0 08	8	0.00
2. Internode borer larvae alone kept under dark	10	0.33	10	0.00
3. Internode borer larvae along with came bits kept under dark	10	0.07	10	0.00
4. Mealy bugs with cane	60	0.31	1	*NT
5. Shoot borer eggs	6	0.00	2	0.00

*NT: Not tested

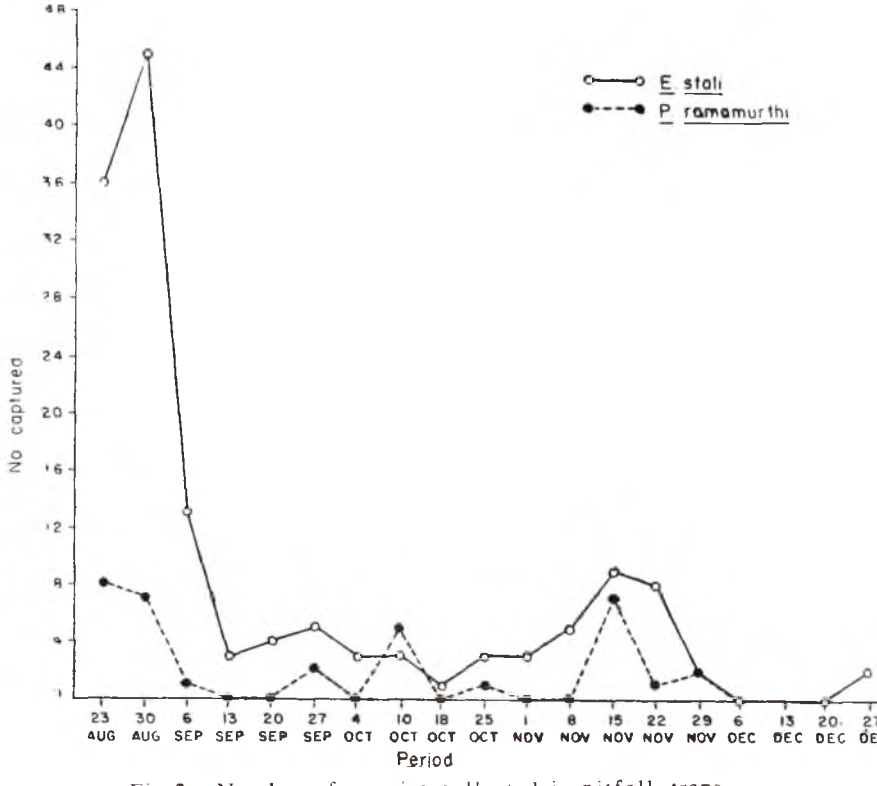

Fig 2. Number of earwigs collected in pitfall traps

was initiated but the populations decreased progressively and during the four weekly observations during December only two individuals of *E. stali* were trapped. The egg clusters as well as nymphs and adults were found on the inner side of the leaf sheath on sugarcane plants more commonly in fields where the moisture level was high,

The study clearly indicates that these two species of earwigs may not play any role in the regulation of borers or mealy bugs in sugarcane ecosystem.

Acknowledgement: We are grateful to Dr. K. MOHAN NAIDU, Director, Dr. H. DAVID, Senior Entomologist, Sugareane Breeding Institute, Combatore for the facilities provided. Our sincere thanks are due to Dr. A. BRINDLE, Commonwealth Institute of Entomology, London for identifying the insects.

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NEW SPECIES OF THE GENUS *TENUIPALPUS* DONNADIEU (TENUIPALPIDAE : ACARINA) FROM INDIA

G. L. SADANA & B. K. GUPTA

Department of Zoology, Punjab Agricultural University, Ludhiana, India 141 004

(Received 14 November 1982)

Four new species of the genus *Tenuipalpus* Donnadieu namely *Tenuipalpus solanensis* sp. nov., *T. guptai* sp. nov., *T. sharmai* sp. nov., and *T. jagatkhanaensis* sp. nov., are described and illustrated. A key to the Indian species is also given.

(Key words: new species of *Tenuipalpus*, mites*)*

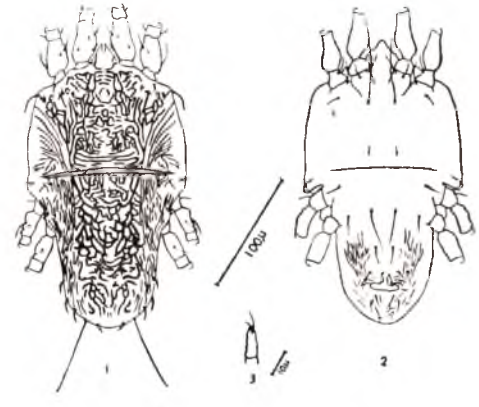
INTRODUCTION

The family Tenuipalpidae includes 21 genera of plant feeding mites (Meyer, 1979). The genus *Tenuipalpus* Donnadieu, 1875 is a large genus of this family of worldwide distribution.

Of about two hundred and seven species of this genus known from the world, only twenty-seven species have been reported from India (Menon, *et al.*, 1971; Dhooria and Sandhu, 1973; Nageshchandra and Channa Basavanna, 1974; Channa Basavanna and Lakkundi, 1976; Maninder and Ghai, 1978 a, b; Sadana and Chhabra, 1980a, b, c; Sadana *et al.*, 1981; Mohanasundaram, 1981; Nassar Ghai, 1981). During an extensive survey of tenuipalpid mites from different plants of economic importance from northern India, four new species of *Tenuipalpus* have been recorded which are described and illustrated here. A key to the Indian species is also given.

The holotypes and paratypes of the new species are deposited in the acarological collections of the Department of Zoology, Punjab Agricultural University, Ludhiana.

1. Tenuipalpus solanensis sp. nov. (Figs. 1—3)



Figs. 1—3. *Tenuipalpus solanensis* sp. nov.

Female: Body 279.50[1] long including rostrum and 168 wide. Palpus 3 segmented, 2nd segment with a long seta, terminal segment with a sensory rod. Rostral shield notched medially. Rostrum reaching upto posterior end of femur I. Propodosoma with strong convolutions medially; thick and oblique striations laterally. Propodosomal setae

1 All measurement are in μm, unless otherwise stated.

3 pairs, I 5, II 5, III narrowly lanceolate, 25.80 long. Eyes two pairs, one pair on each side. Humeral setae 1 pair, minute. Hysterosoma with strong convolutions medially: broken and longitudinal striations laterally; with a pair of pores. Dorsocentral setae 3 pairs, simple, minute. Lateral setae 6 pairs lanceolate, I 6 II 8.60, III 12.90, IV 12.90 V 86, flagellate, VI 12.90 long respectively. Lateral setae II falls short of distance between setae II and III, setae III and IV reach the bases of setae IV and V respectively.

Gnathosoma without any setae ventrally. Propodosoma without striations ventrally. Medioventral propodosomal setae 1 pair, 55 90 long; anterior medioventral metapodosomal setae 1 pair, 8.60 long; posterior medioventral metapodosomal setae 1 pair, 51.60 long and cross the bases of ventral shield setae; ventral shield setae 1 pair, 21.50 long; genital shield setae 2 pairs, each being 15 long; anal setae 2 pairs. All setae simple.

Legs 4 pairs, segment wrinkled. Setae and selenidia (in paranthesis) on legs I—IV: Coxae 2-2-1-1: trochanters 1-1-2-1: femora 4-4-2-1: genua 2-2-0-0: tibiae 5-5-3-3: tarsi 7(1)-7(1)-4-4.

Male: Note known

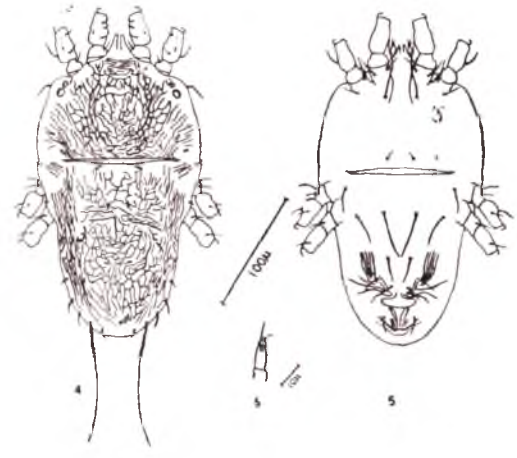
Holotype: ♀, encircled on slide No. 91. ex. *Punica granatum* L., 22. vi. 1981, Solan, Coll. Bimal Kumar Gupta.

Paratype: 2 ♀♀ on same slide with same collection data.

Remarks: Tenuipalpus solanensis sp. nov. resembles *T. lustrabalis* (Chaudhri, 1971) but differs from it in rostral shield structure; pattern of convolutions on dorsum and in having long lateral setae IV which reach the bases of setae V.

This species is named after the locality.

2. **Tenuipalpus guptai** sp. nov. (Figs. 4—6)



Figs. 4—6. *Tenuipalpus Guptai* sp. nov.

Female: Body 292.40 long including rostrum and 172 wide. Palpus 3 segmented, 2nd segment with a long seta, terminal segment with a sensory rod. Rostral shield notched medially. Rostrum reaching upto middle of femur I. Propodosoma with transverse broken striations anteromedially: irregular and broken reticulations medially; a few complete reticulations mediolaterally and oblique striations laterally. Propodosomal setae 3 pairs, I 4, II 4, III narrowly lanceolate, 25.80 long. Eyes two pairs, one pair on each side. Humeral setae 1 pair, each 6.45 long. Hysterosoma with irregular and broken reticulations medially a few of these forming complete reticulations; lateral sides with broken longitudinal striations; with a pair of pores. Dorsocentral setae 3 pairs, simple, each 6.45 long. Lateral setae 6 pairs, lanceolate with small serrations, I 4.30,

II 8.60, III 12.90, IV 10.75, V 103.20 flagellate and VI 10.75 long respectively. Lateral setae II falls short of distance between setae II and III, setae III and IV reach the bases of setae IV and V respectively.

Ganthosoma without any setae ventrally. Propodosoma without striations ventrally. Medioventral propodosomal steae 1 pair, 77.40 long; anterior medioventral metapodosomal setae 1 pair, 8.60 long; posterior medioventral metapodosomal setae 1 pair, 47.30 long and reaching the bases of ventral shield setae; ventral shield setae 1 pair, 21.50 long; genital setae 2 pairs, each being 17.20 long; anal setae 2 pairs. All setae simple.

Legs 4 pairs, segments wrinkled. Setae and solenidia (in parantheses) on legs I—IV: Coxae 2-2-1-1; trochanters 1-1-2-1; femora 4-4-2-1; genua 2-2-0-0; tibiae 5-5-3-3; tarsi 5(1)-5(1)-4-4.

Male: Not known

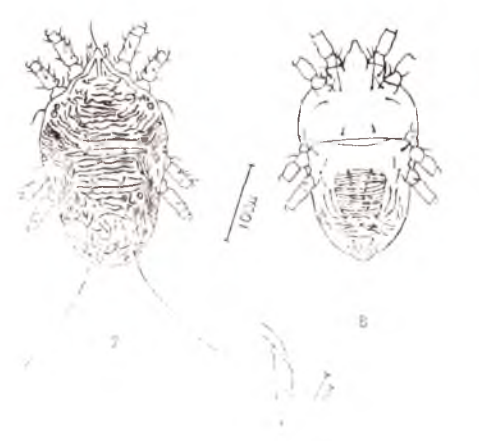
Holotype: ♀, encircled on slide No. 91, ex. *Punica granatum* L., 22.vi.1981, Solan, Coll. Bimal Kumar Gupta.

Remarks: Tenuipalpus guptai sp. nov. resembles *T. placitus* (Chaudhri, 1971) but differs from it in striations pattern of dorsum and venter and in having longer lateral setae III which reach the bases of setae IV.

This species has ceen named after the collector.

3. Tenuipalpus sharmai sp. nov. (Figs. 7—9)

Female: Body 290 long including rostrum and 160 wide. Palpus 3 segmented, 2nd segment with a long seta, terminal segment with a sensory rod. Rostral shield notched medially. Rostrum reaching upto middle of femur I. Propodosoma with transverse, thick and



Figs. 7—9. *Tenuipalpus sharmai* sp. nov.

wavy striations medial, oblique and wavy laterally. Propodosomal setae 3 pairs, I 10, II 15, III 20 long respectively. Eyes two pairs, one pair on each side. Humeral setae 1 pair, each seta 10 long. Hysterosoma with transverse, thick, wavy striations medially but irregular posteriorly, oblique laterally. Central setae 3 pairs, simple, all being 10 long Lateral setae 6 yairs, I 8, II 10, III 10, IV 10, V 130 flagellate, VI 10 long respectively. Lateral setae IV reach the bases of setae V.

Gnathosoma with a pair of setae ventrally. Propodosoma without striations ventrally. Medioventral propodosomal setae 1 pair, 70 long; anterior medioventral metapodosomal setae 1 pair, 10 long; posterior medioventral metapodosomal setae 1 pair, 50 long and reach the bases of genital shield setae. Transverse striations on genital plate, ventral shield setae 1 pair, 15 long; genital shield setae 2 pairs, each being 15 long; anal shield setae 2 pairs. All setae simple.

Legs 4 pairs, segment wrinkled. Setae and solenidia (in paranthases) on legs I—IV: Coxae 2-2-1-1; trochanters

1-1-2-1; femora 4-4-2-1; genua 3-3-1-0; tibiae 5-5-3-3; tarsi 5(1)-4(1)-4-4.

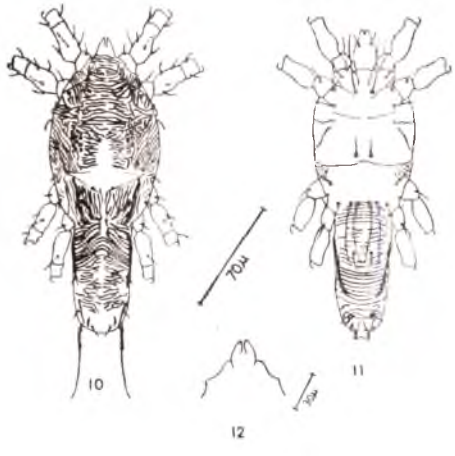
Male: Not known.

Holotype: ♀, encircled on slide No. 71, ex. *Helianthus annus*, 20. vii, 1981, Punjab Agricultural University, Ludhiaua, Coll. Rajinder Sharma.

Remarks: Tenuipalpus sharmai sp. nov. kxys out near *T. protectus* (Meyer, 1979) but differs from it in striations pattern of dorsum and venter; leg chaetotaxy and in having the second pair of propodosomal setae shorter than third pair. It also resembles *T. lunatus* (Mayer, 1979) but differs from it in striations pattern of venter; leg chaetotaxy and non-distinction between venral and genital shields. It also resembles *T. mustus* (Chaudhri, 1972) but differs from it in having different striations pattern on dorsum and venter and in possing larger third pair of propodosomal setae.

This species is named after the collector.

4. Tenuipalpus jagatkhanaens is sp. nov. (Figs. 10—12)



Figs. 10—12. *Tennipalpus jagatkhanaens* sp. nov.

Male: Body 240.80 long including rostrum and 90.30 wide. Palpus one segmented with a terminal seta. Rostral shield notched medially with 2 prominent lateral lobes on each side. Rostrum extending beyond the middle of femur I. Propodosoma with thick transverse, broken striations medially: oblique and wavy striations postero-laterally: distinct areolae mediolateraqlly and without striations postero-medially. Propodosomal setae 3 pairs, I 5, II 5, III being 10.75 long, eyes two pairs, one pair on each side. Humeral setae 1 pair, each 8.60 long. Hysterosoma with broken longitudinal striations anteriorly; area in front of central setae I without striations; transverse striations between central setae II and III: broken transverse striations posterior to central setae III; posteriormost area without striations. Dorsocentral setae 3 pairs, I 6.45, II 4.30, III 4.30 long respectively. Lateral setae 5 pairs, I 8.60, II 6.45, III 8.60, IV 77.40, flagellate and serrate, V 8.60 long respectively.

Gnathosoma with a pair of ventral setae. Ventrally, propodosoma with 1—2 transverse and an oblique striations. Hysterosoma with transverse striations posterior to bases of posterior medioventral metapodosomal setae, flanking broken transverse striations medially; area anterior to coxae III with oblique striatcons. Medioventral propodosomal setae 1 pair, 51.60 long; anterior medioventral metapodosomal setae 1 pair, 10.75 long; posterior medioventral metapodosomal setae 2 pairs, inner 38.70 and outer 34.40 long respectively, both the pairs cross the basesof ventral shield setae. Genital areawith broken transverse striations anteriorly and transverse striations posteriorly Ventral shield setae 1 pair, 10.75 long; genito-anal setae 4 pairs.

Legs 4 pairs, segments wrinkled. Setae and solenidia (in parantheses) on legs I—IV: coxae 2-2-1-1; trochanters 1-1-1-1, femora 4-3-2-1, genua 3-3-1-0 tibiae 4-4-2-2; tarsi 7(1)-7(1)-6-6.

Female: Not known

Holotype: ♂, encircled, on slide No. 92, ex *Achras sapota*, 13.iii.1980, Jaget Khana (Nalagarh), Coll. S. C. Chhabra.

Paratype: ♂ on same slide with same collection data.

Remarks: Tenuipalpus jagatkhanaensis sp. nov. is close to *Tenuipalpus punjabensis* (Maninder & Ghai) but differs from it in reticulations of dorsum and venter, leg chaetotaxy and absence of lateral pores.

This species is named after the locality.

KEY TO THE INDIAN SPECIES OF *TENUIPALPUS* DONNADIEU

1. Hysterosoma with four pairs of non-flagellate caudolateral setae 2
   Hysterosoma with three pairs of non-flagellate caudolateral setae 23
2. Hysterosoma differentiated to form an expansion anterior to coxae III ..... *T. micheli* Lawrence, 1943
   Hysterosoma without such differentiated expansion 3
3. Gnathosoma without ventral setae..... 4
   Gnathosoma with a pair of ventral setae 8
4. Dorsum with strong convolutions.... ..... *T. solanensis* sp. nov.
   Dorsum without strong convolutions: either with reticulations or striations ...5
5. Dorsum with strong reticulations or striations..... 6
   Dorsum with a few thick or thin reticulations 7
6. Dorsum with strong reticulations ..... *T. punicae* Pritchard & Baker, 1958
   Dorsum with thick striations but middle of propodosoma with thick reticulations ..... *T. caudatus* (Duges), 1875
7. Dorsum with a few thick reticulations; hysterosoma with a pair of pores ..... *T. guptai* sp. nov.
   Dorsum with a few thin reticulations; hysterosoma without such pores ..... *T. abohariensis* Sadana & Chhabra, 1980
8. Dorsocentral hysterosomal setae one pair ..... *T. granati* Sayed, 1946
   Dorsocentral hysterosomal setae three pairs 9
9. Anterior medioventral metapodosomal setae two pairs 10
   Anterior medioventral metapodosomal setae one pair 12
10. Setae on genua I-IV: 2-2-1-0; setae on tibiae I-IV: 4-4-4-4 11
    Setae on genua I-IV: 3-2-1-0; setae on tibiae I-IV: 4-4-3-2..... *T. coimbatorensis* Mohanasundaram, 1981
11. Setae on femora I-IV: 4-4-2-1 ..... *T. pernicis* Chaudhri, Akbar & Rasool, 1974
    Setae on femora I-IV: 5-5-2-2..... .... *T. cissampelosa* Maninder & Ghai, 1978b
12. Posterior medioventral metapodosomal setae one pair, dorsum with transverse and cross striations or reticulations.... 13
    Posterior medioventral metapodosomal setae two pairs; dorsum with or without polygonal reticulations 18
13. Dorsum with transverse and cross striations 14
    Dorsum with reticulations .... *T. ghaii* Mohanasundaram, 1981
14. Dorsum with thin transverse striations; third pair of prodosomal setae relatively short to minute ..... *T. mustus* Chaudhri, 1972
    Dorsum with thick transverse and cross striations; third pair of propodosomal setae somewhat lengthened..... 15
15. Palpus 4 segmented; second palpal segment with false segmentation..... ..... *T. yousefi* Nassar and Ghai, 1981
    Palpus 3 segmented, second palpal segment without false segmentation..... 16

16. Terminal palpal segment with single solinidia; setae on tibiae I–IV: 5-5-3-3 ..... *T. sharmai* sp. nov.

Terminal palpal segment with two solinidia; setae on tibiae I-IV: 4-4-3-3... 17

17. Setae on femora I–IV: 3-3-2-1; setae on genua I–IV: 1-1-0-0 ..... *T. malligai* Mohanasundaram, 1981

Setae on femora I–IV: 3-3-2-2; setae on genua I–IV: 3-2 1-1 .... *T. laminasetae* Mohanasundaram, 1981

18. Dorsum with polygonal reticulations... 19

Dorsum without polygonal reticulations.. 22

19. Second dorsolateral hysterosomal pair reaching upto the base of third dorsolateral pair ..... *T. dimensus* Chaudhri, 1971

Second dorsolateral hysterosomal pair not reaching upto the base of third dorsolateral pair 20

20. Rostral shield reaching upto the anterior end of trochanter I....*T. pyruseae* Maninder & Ghai, 1978b

Rostral shield reaching upto the middle of femur I 21

21. Setae on trochanters I–IV: 1-1-2-1; on tibiae 5-5-3-3 ..... *T. ludhianaensis* Sadana & Chhabra, 1980

Setae on trochanters I–IV: 1 1-2-2; on tibiae 4-4-3-3..... *T. pruni* Maninder & Ghai, 1978b

22. Dorsal body setae lanceolate 23

Dorsal body setae not lanceolate 25

23. Dorsum with broad ridges and furrows ..... *T. ixorae* Maninder & Ghai, 1978b

Dorsum with simple but prominant lining or reticulate pattern in the middle and elongated wavy diagonal lines on the sides 24

24. Dorsum with simple but prominant linings; femora I–IV: 5-5-2-2 ..... *T. fici* Maninder and Ghai, 1978b

Dorsum with reticulate pattern in the middle and elongated wavy diagonal lines on the sides ..... *T. tectonae* Mohanasundaram, 1981

25. Second palpal segment as long as broad 26

Second palpal segment considerably longer than broad 27

26. Rostral shield extending beyond the middle of femur I ..... *T. faresianus* Maninder & Ghai, 1978b

Rostral shield extending slightly above the anterior end of trochanter I ... *T. acacii* Maninder & Ghai, 1978b

27. Second palpal segment with false segmentation; propodosoma with semicircular linings in the middle ... *T. indicus* Maninder & Ghai, 1978b

Second palpal segment without false segmentation; propodosoma with irregular pattern of transverse and longitudinal reticulations ... *T. amygdalusce* Maninder & Ghai, 1978b

28. Posterior medioventral metapodosomal setae one pair ... *T. lalbaghensis* Channa Basavanna & Lakkundi, 1976

Posterior medioventral metapodosomal setae more than one pair 29

29. Posterior medioventral metapodosomal setae two pairs 30

Posterior medioventral metapodosomal setae three to four pairs ..... *T. crassus* Andre, 1953

30. Setae on femora I–IV: 5-5-3-3; on genua 2-2-1-0; on tibiae 4-4-3-3 *T. punjabensis* (Maninder & Ghai) 1978a

Setae on femora I–IV: 4-3-2-1; on genua 3-3-1-0; on tibiae 4-4-2-2 ..... *T. jagatkhanaensis* sp. nov.

Acknowledgements: The authors are thankful to Dr. S. S. Guraya, Professor & Head, Department of Zoology, Punjab Agricultural University, Ludhiana, for providing laboratory facilities and I C A R for financing the scheme to carry out the taxonomic work on Tetranychoid mites. The help rendered by S C Chabra and Rajinder Sharma is gratefully acknowledged.

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REGULAR OCCURRENCE OF INTRA-INDIVIDUAL VARIATIONS OF *X* CHROMOSOMAL ELEMENTS AND THEIR SUBSEQUENT REGULARIZATION IN A LARGID BUG, *LOHITA GRANDIS* GRAY

G. K. MANNA & S. DEB-MALLICK[1]

Department of Zoology, University of Kalyani, Kalyani, West Bengal, India 741 235

(Received 26 February 1984)

A peculiar case of the unstable *X* chromosome number and size in the gonial metaphases of the same female and male, and also during meiosis upto the primary spermatocyte metaphase and the subsequent elimination of all the extra *X*'s barring one, possibly the original *X*, during the second spermatocyte division has been observed in the largid bug, *Lohita grandis*. The species seemed to have basically *XO : XX* sex chromosome mechanism at fertilization but the *X* chromosomal number became variable and correspondingly their sizes diminished in different cells of the same individual as the development proceeded in both sexes leading to the unstable condition of the *X* chromosome. In females the oogonial number appeared basically to be 16 $(12A + 2m + XX)$ of which 12 autosomes and 2*m* chromosomes were constant in every cell but the sizes and correspondingly the number of the two *X* chromosomes varied upto 7 leading to the variable oogonial numbers in different plates of the same individual. The spermatogonial number in every male seemed basically 15 $(12A + 2m + XO)$ but it varied upto 23 depending on the higher number of *X* elements in different plates of the same individual while the number of 12 autosomes and 2*m* chromosomes remained unaltered. During spermatogenesis the variable sizes and numbers of the *X* chromosome were present upto metaphase I in every male but the metaphase II contained invariably 8 elements consisting $6A + m + X$ indicating thereby all but one *X*, possibly the original *X*, underwent dissolution. The manner of dissolution of the extra *X* elements between late metaphase I and early anaphase I was, however, untraceable. Anaphase II was reductional for the single *X* giving rise to two types of sperms having $6A + m + X$ and $6A + m$. Though not studied, the elimination of the extra *X* elements has also been suspected in females during oogenesis thereby every mature egg would contain $6A + m + X$ which on fertilization by two types of sperms, males would start development with 15 and females with 16 chromosomes. After a few cleavage divisions *X* chromosomal anomaly with regard to its number and size originated which led male and female individuals to develop with anomalous *X* chromosomal numbers and sizes. This was rectified at metaphase II in males and at some stage of oogenesis in females. The origin of the extra *X* chromosomal elements and the reduction in size have been explained as due to chromatin elimination rather than fragmentation of the original *X* chromosome.

(Key words: regular increase and elimination of extra *X* chromosomal elements, largid bug, *Lohita grandis*, Largidae : Heteroptera).

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1 Present Address: Department of Zoology, North Bengal University College at Raiganj, Raiganj 733 134, West Dinajpur, West Bengal.

INTRODUCTION

Earlier, BANERJEE (1959) in *Lohita* (as *Macroceroea*) *grandis* reported the spermatogonial number basically of 15 chromosomes but at metaphase I different plates of the same male individual contained 7 autosomal bivalents and variable number of *X* chromosomes ranging between 1 and 7 while at metaphase II there were only 7 autosomes and a single *X* with the elimination of the fragmented extra *X* elements. He (BANERJEE, 1959) also found 16 chromosomes in the oogonial complement and opined that in males during first spermatocyte the single *X* underwent variable number of fragmentation giving rise to plates with different *X* chromosomal number but all except one *X* survived and the other disintegrated at metaphase II. During our recent survey of the gonial numbers and behaviour of chromosomes at spermatogenesis (MANNA & DEB-MALLICK, 1981a), we came across a population of *L. grandis* at Kalyani. It was about 50 km north of the place of collection of BANERJEE (1959). The present study revealed a good amount of fundamental difference with that of BANERJEE (1959) indicating that the species could be polymorphic for a pair of *m* chromosomes, instability of gonial number in different plates of the same male and female individuals etc. Moreover, in the present study an attempt has been made to unveil the mechanism of the origin of the anomalous number of *X* chromosomal elements and its subsequent rectification at metaphase II aided by the metrical data of chromosomes of metaphase I. The significance of the intra-individual anomalous distribution of *X* chromosomal element in both sexes has been discussed.

MATERIAL AND METHODS

Thirty females, 125 males and 12 fourth instar male nymphs of *Lohita* (= *Macroceroea*) *grandis* Gray (Larginae, Largidae, Heteroptera) collected from the host plant, *Trewia nudiflora* at Kalyani, West Bengal constituted the material of the present study. Ovaries of each female, and testes of each adult male and of nymph were fixed separately in acetic-alcohol mixture (1 : 3), squashed in 45% acetic acid solution between the slide and the cover-glass and stained mostly in iron-alum haematoxylin and some in Feulgen stain. The metrical data of chromosomes of different first division metaphase plates were scored following the method described by MANNA (1951).

OBSERVATION

Fifteen out of 30 females of *L. grandis* had few oogonial metaphases in which the number ranged between 16 and 21 chromosomes (Figs. 1—4, 25, 26). The minimum number of 16 chromosomes (Figs. 1, 15) has been taken as the basic one for various considerations (*vide infra*), in which a pair of very minute *m* chromosomes could be demarcated from the rest while one pair of relatively large *X* chromosomes was not so distinctly demarcable from the 12 other gradually seriated autosomes of more or less similar size. In oogonial complement with 21 chromosomes (Figs. 4, 26) there were 7 *X* chromosomal elements of about the size of the *m*-pair when the individual identity of the latter became obscure unless the *X* elements showing sometimes a little staining difference. In the same individual plates having more than 16 chromosomes there were variable number of *X* chromosomal elements, the sizes of which were reduced as the number of *X* elements was increased while 12 autosomes and two *m*-chromosomes remained constant. Thus there were 3*X*'s in 17 chromosomes bearing plate (Fig. 2), 4*X*'s in 18 chromosomes

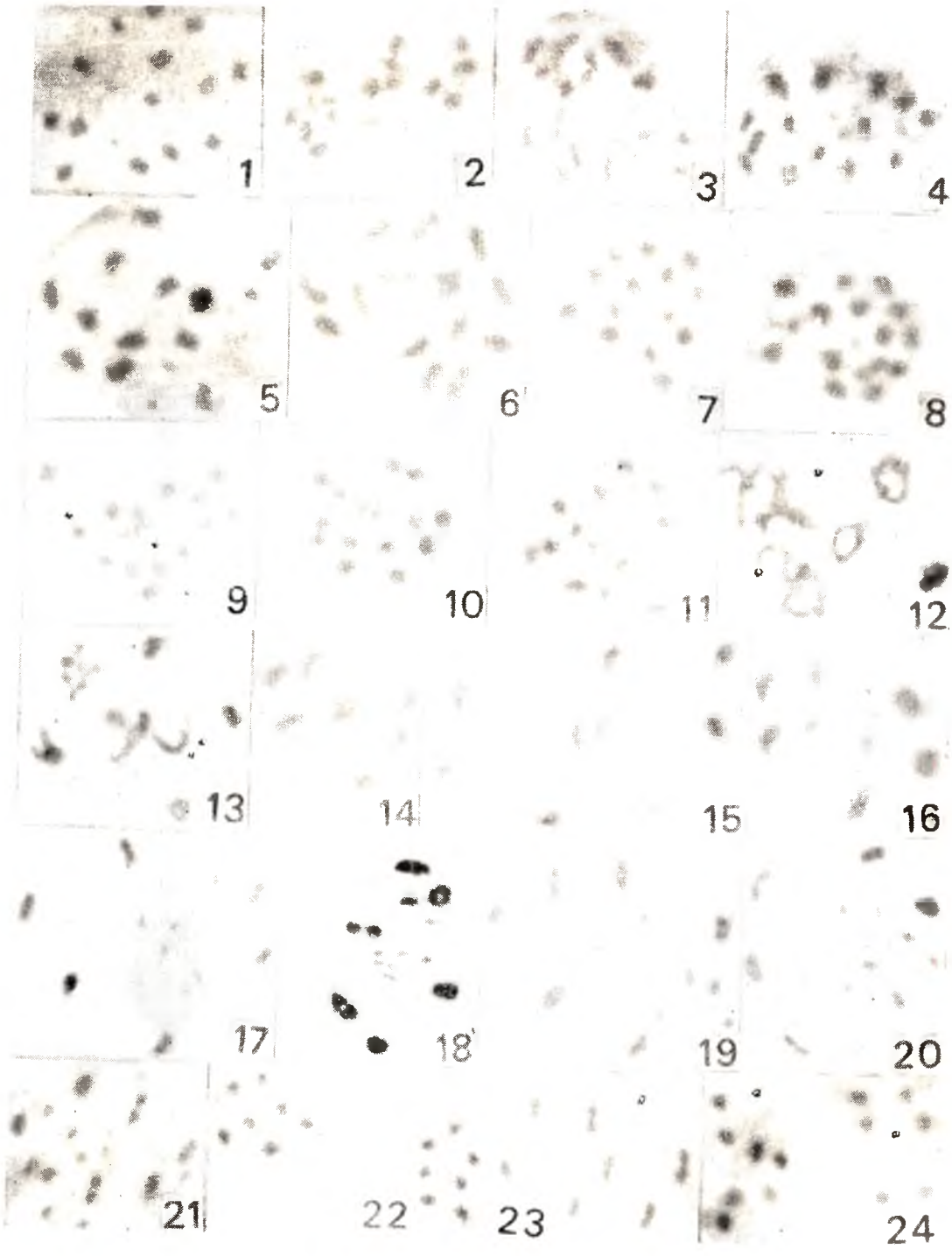
(Fig. 3), 7*X*'s in 21 (Fig. 4) and it was expected to have 10*X*'s in 24 chromosome bearing plate but all numbers were not encountered for paucity of division if some unknown limiting factor was not operating. Stages of oocytic division other than some early prophase I were not encountered possibly for their shorter duration and conditional periodic occurrence as found in other insects.

In the testes of all 125 adult males and of some nymphs early and full spermatogonial metaphase complements in an individual had variable numbers ranging between 15 and 23 chromosomes (Figs. 5—11, 27—33). The frequency of metaphases having different number in different individuals could not be specifically determined as the number was very low and all plates were not clear enough to determine chromosome numbers accurately. Anyhow in every individual metaphase complements with chromosome numbers ranging from 15 to 23 were not encountered. Plates with 15 chromosomes were taken as the basic one which contained 13 gradually seriated medium to small sized chromosomes and a pair of demarcable minute *m*-chromosomes (Figs. 5, 27). Occasionally in some plate one slightly large ovoid chromosome was seen which could be single *X* (Fig. 5) but mostly size distinction between the single *X* and other 12 autosomes was not appreciable (Fig. 27). Similar erratic behaviour was seen in a few spermatogonial complements with 16 chromosomes which contained a somewhat large element, 13 medium to small and a pair of *m*-chramosomes (Fig. 6) but in other plates with 16 chromosomes no such distinguishable large element was seen (Figs. 7, 28). Anyhow, as the number of *X* chromosomal elements increased correspondingly their sizes were diminished and this was the sole factor for the variation in the spermatogonial numbers in different plates of the same individual because 12 autosomes and two *m*-chromosomes remained constant while 15 chromosomes bearing plate had one *X* (Figs. 5, 27), 16 had 2*X*'s (Figs. 6, 7), 17 had 3*X*'s (Fig. 28), 18 had 4*X*'s (Fig. 8), 19 had 5*X*'s (Figs. 9, 29); 20 had 6*X*'s (Figs. 10, 30), 21 had 7*X*'s (Fig. 11), 22 had 8*X*'s (Fig 31) and 23 had 9 *X*'s (Fig. 32) and no number beyond this was encountered. It was noted that the sizes of the *X* elements were palpably reduced when their number was 5 or more (Figs. 9, 10, 29—32). In plates with 23 chromosomes (Fig. 32) or so the size of the *X* elements appeared even smallar than the *m*-chromosomes. In such a situation the *m*-pair could only be demarcated if there was sometimes slight staining difference.

During spermatogenesis the early primary prophase nuclei contained diffusely stained thin thread-like autosomes and one or more Feulgen positive elements mostly lying close to the nuclear membrane. The deeply stained mass in all probability represented the sex chromatin. The diplotene stage (Figs. 12, 33) contained invariably 6 diffusely stained autosomal bivalents, 2 *m*-chromosomes paired or free and variable number of deeply stained *X* elements showing a tendency of lying close or remaining still fused into a mass (Fig. 12). The number of *X* chromosomal elements in different diakinesis nuclei was also variable but 6 autosomal bivalents and two *m*-chromosomes were constantly present (Figs. 13, 34). That the number of *X* chromosomal elements ranging from 1 to 9 in different plates of metaphase I in the same male individual while 6

autosomal bivalents and the *m*-pair remaining constant could clearly be followed because of their favorable independent disposition (Figs. 14—21, 35—41). At metaphase I, a characteristic disposition of chromosomes could be made out. The *m*-pair and one or more *X* elements were lying inner to the circle formed by 6 autosomal bivalents (Figs. 17—20, 38—40) at the equatorial region but the arrangements were sometimes disturbed by the artificial squashing pressure. As found in oogonial and spermatogonial complements intra-individual variation of *X* chromosomal number, so also in different plates of first spermatocyte metaphase the number of the *X* chromosomal elements was increased and correspondingly their sizes were reduced. With a view to finding out if the increase of the *X* chromosomal elements could be correlated with the fragmentation of the originally single *X* element as represented in 15 chromosome bearing plate to give rise to as may as 9 in 23 chromiosome bearing plate, Metrical study of chromosomes of different plates of metaphase I bearing 1 *X* to 9 *X*'s was carried out. The volume expressed by the average number of squares covered in inch scale graph paper after further magnification of the camera lucida drawing by its epidiascope projection (MANNA, 1951) was A_1 (Autosomes No. 1) = average 196 squares ranging betweeu 124 and 282; A_2 = average 178 squares ranging between 123 and 260; A_3 = average 175 squares ranging between 93 and 220; A_4 = average 152 squares ranging between 93 and 213; A_5 = average 140 squares ranging between 86 and 190 and A_6 = average 130 squares ranging between 73 and 173 while the *m* = average 52 squares ranging between 36 and 72. On the other hand the average number of squares covered by the *X* element in different plates of metaphase I was 1 *X* = 205; 2*X*'s = 152 and 135; 3*X*'s = 72, 60 and 55; 4*X*'s = 60, 35, 35 and 28; 5*X*'s = 83, 75, 62, 55, and 50; 6*X*'s = 85, 63, 55, 50, 48 and 36; 7*X*'s = 73, 52, 50, 45, 42, 41 and 40; 8*X*'s = 60, 41, 35, 32, 32, 28, 28 and 24 and 9*X*'s = 55, 34, 32, 31, 30, 29, 28, 26 and 23. The total number of squares covered by more than one *X* present in different plates was *XX*'s = 287; 3*X*'s = 187; 4*X*'s = 148; 5*X*'s = 325; 6*X*'s = 337; 7*X*'s = 343; 8*X*'s = 280 and 9*X*'s = 288 while 1X =

Fig. 1. 16 chromosomes; Fig. 2. 17 chromosomes; Fig. 3. 18 chromosomes; and Fig. 4. 21 chromosomes in oogonial metaphases; Fig. 5. 15 chromosomes; Fig. 6. 16 chromosomes; Fig. 7. 16 chromosomes; Fig. 8. 18 chromosomes; Fig. 9. 19 chromosomes; Fig. 10. 20 chromosomes and Fig. 11. 21 chromosomes in spermatogonial metaphases; Fig. 12. Late diplotene, *X* elements formed a mass; Fig. 13. Seven *X* elements interconnected and *m*-chromosomes in pair at diakinesis stage; Fig. 14. Metaphase I, *X* chromosomal mass at the centre encircled by 6 bivalents and the *m*-pair; Fig. 15. Metaphase I, 3 *X* elements and the *m*-pair inner to the autosomal ring; Fig. 16. Metaphase I, 4 *X* elements and the *m*-pair in the inner area; Fig. 17. 5 *X*-elements and the *m*-pair not sharply demarcable; Fig. 18. 6 *X* elements appeared smaller than the *m*-pair encircled by 6 autosomal bivalents; Fig. 19. Seven *X* elements and the *m*-pair not sharply demarcable Fig. 20. Eight *X* elements and *m*-pair not demarcable; Fig. 21. Metaphase I, 9 *X* elements and the *m*-pair not distinguishable; Fig. 22. Anaphase I each daughter nucleus with 8 elements; Fig. 23. Metaphase II, the single *X* seemed to be at the centre of 7 autosomes and one *m* chromosome; Fig. 24. Anaphase II, one daughter half with 8 elements and the other with 7 elements.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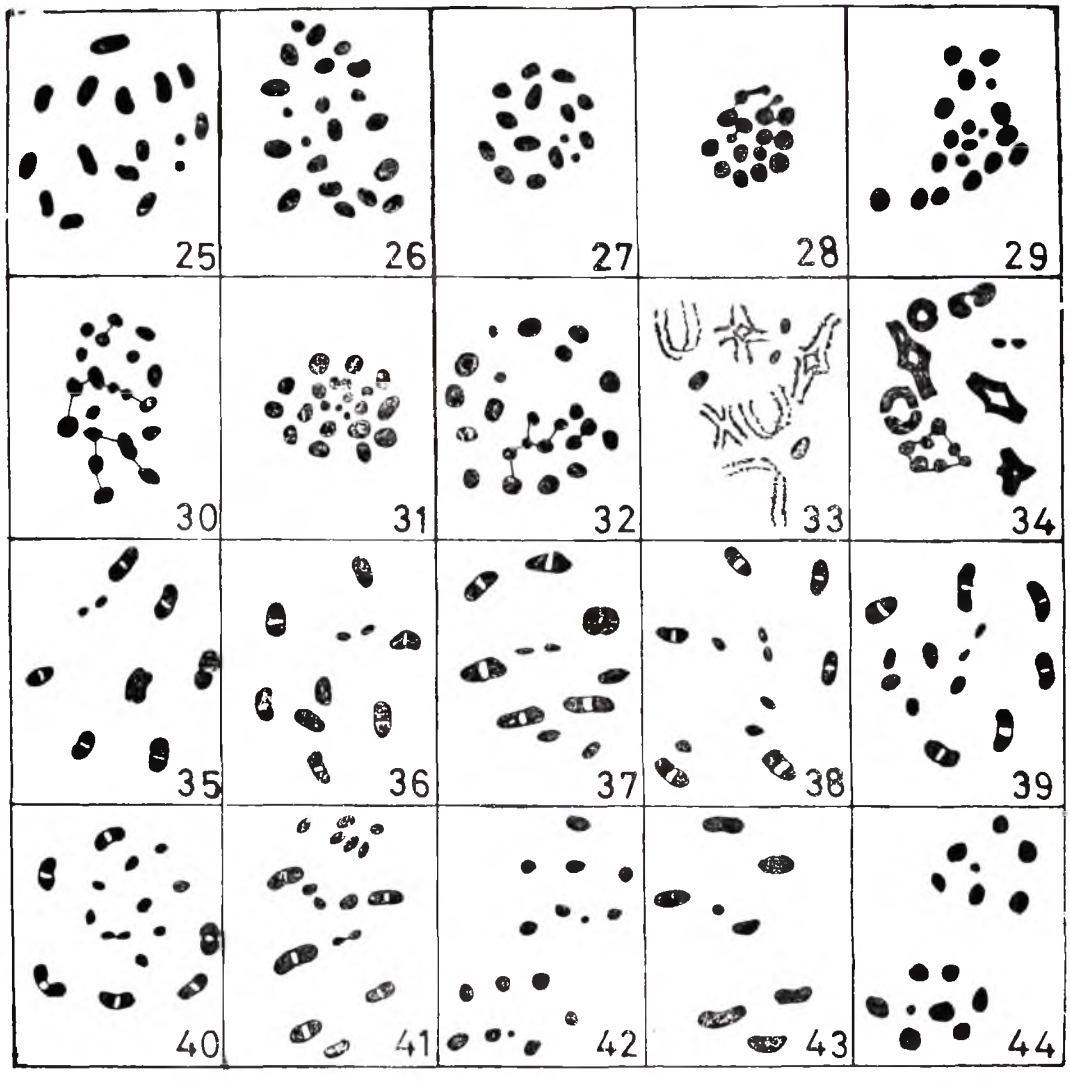

Camera lucida drawings × ca 2000. Fig. 25. 16 chromosomes and: Fig. 26. 21 chromosomes of oogonial metaphase; Fig. 27. 15 chromosomes; Fig. 28. 17 chromosomes; Fig. 29. 19 chromosomes; Fig. 30. 20 chromosomes; Fig. 31. 22 chromosomes and Fig. 32. 23 chromosomes of spermatogonial metaphases; Fig. 33. Late diplotene with 6 bivalents, 2*m* and 2*X* chromosomes; Fig. 34. Diakinesis with 6 bivalents, one *m*-pair and 7 *X* elements connected by thin connnection; Fig. 35. Metaphase I with one *X*; Fig. 36. with Metaphase I with 2 *X* elements; Fig. 37. Metaphase I with 3 *X* elements; Fig. 38. with 4 *X*; Fig. 39. with 5 *X*; Fig. 50. with 8*X* and; Fig. 41. with 9 *X* elements; Fig. 42. Anaphase I each daughter half with 8 elements; Fig. 43. Metaphase I with single *X* and the *m* chromosomes at the central part; Fig. 44. Anaphase II, one pole with 8 and the other with 7 elements.

205, The metrical data did not indicate unquestionably the occurrence of the higher number of *X* elements by gradual fragmentation of the original single *X* firstly because the values were erratic when *X* elements of lower and higher number were compared and secondly the origin of the higher number of *X* elements could also be reconciled with sex chromatin elimination hypothesis and so on. Anyhow the metrical data did not give any univocal support on the origin unstable number of *X* chromosomes either by fragmentation or by chromatin elimination in different cells. The frequency of metaphase plates with different number of *X* chromosomes in different individuals was not determined because the numbers of plates were not adequate. Unlike other heteropteran bugs in general anapase I in *L. grandis* was peculiar because instead of undergoing equational division the extra *X* elements un-noticably went in dissolution and one *X* element very likely the original *X* underwent equational division giving rise to each daughter half with 8 elements viz. *6A*+*m*+*X* (original?) (Figs. 22, 42). How the extra *X* elements present in good number of plates of metaphase I underwent dissolution remained unresolved because in clear anaphase I plates no laggards or degenerating elements were seen. Rarely a chromosome bridge was found. It has been suspected that the elimination or dissolution of all but one *X* chromosomes took place between and of metaphase I and very early anaphase I. That all but one *X* element underwent dissolution during anaphase I was confirmed at metaphase II which contained nothing but 8 elements comprising 6 autosomes, one *m*-chromosomes and an undemarcable *X* about the size of the large autosome (Figs. 23, 43). At metaphase II unlike metaphase I the number of chramosomal elements did not vary at all and it was always 8 with the single *X* sometime forming an accessory plate when it was individually identifiable. The size of the *X* element varied slightly from plate to plate which could be indicative that it was regaining its original form because it underwent numerical changes in earlier stages. Anaphase II was reductional for the single *X* chromosome as found orthodoxically for Heteroptera. In favourable plates of anaphase II the two types of chromosomal distribution in the to daughter nuclei, one with *6A*+*m*+*X* and the other with *6A*+*m* could clearly be seen (Figs. 24, 44) indicating thereby two kinds of sperms were formed likely in equal number. On the same basis it could be surmised that in females the anomalous number of *X* elements found in original count were eliminated during some oocyte division and eggs contained only 8 elements, *6A*+*m*+*X*.

DISCUSSION

BANERJEE (1959) in his study of the chromosomes of *L. grandis* did not find clearly the presence of a pair of *m*-chromosomes though this was evident in our material. The presence of the *m*- pair was very much expected because the family Largidae has been cytologically characterized by the presence of *m*-pair while its allied family Pyrrhocoridae for lacking them (UESHIMA, 1979; MANNA, 1982, 1983) exept *Iphita limbata* (MANNA & DEB-MALLIK, 1981b). In *L. grandis* the two *m*-chromosomes showed their characteristic behaviour as they were minute in size, heteropycnotic in behaviour, invariably associated in pair and occupying the central area of the spindle sharing with the sex chromosomal elements and surrounded by autosomal bivalents at metaphase I and also in

metaphase II and so on (MANNA, 1982, 1983). Of course in *L. grandis* it could be confused with smaller *X* chromosomal elements in gonial complements of both sexes and in first spermatocyte prophase and metaphase. Both *X* and *m* were of similar size and their distinguishing features were not obvious but there was no scope of such confusion to identify the *m* chromosomes in plates with 15 or 16 chromosomes and specially in all complements of metaphase II. Therefore, it might be concluded that the two populations of *L. grandis*, one studied earlier by BANERJEE (1959) and the other presently by us were polymorphic for the *m*-chromosomes if BANERJEE (1959) did not fail to identify them specifically for some reasons stated above. Possibly BANERJEE (1959) could not realize the importance of the occurrence of *m*-pair in relation to cytotaxonomy of Largidae and he was not critical in his study to identify specifically the *m*-pair. Therefore, it is desirable to re-examine cytologically the population of *L. grandis* studied by BANERJEE (1959) before making a definite claim of the absence of the *m*-pair in the said population.

BANERJEE (1959) failed to observe intra-individual variation of *X* chromosomal number in females like that of males because of his limited attempt and he was also not critical to examine different possibility for the origin of the anomalous number of *X* chromosomal elements in gonial and first spermatocyte cells of the same male individuals in his materials. Anyhow, in the present study it was clearly found the intra-individual variation of *X* chromosomal elements in gonial cells of both sexes, upto 7 in females (might be even more) and 9 in males. The variation of the *X* chromosomal number was followed in different stages of first spermatocyte division which was subsequently reduced to only one *X* in stages of second spermatocyte division. There would be few important points associated with the anomalous and later regular behaviour of the single *X* chromosome: (a) What could be the mechanism of increase in the basic number of single *X* in male and 2's *X* in females leading to the mosaic condition of *X* chromosomal elements in gonadal cells and in primary spermatocyte and its significance? (b) When the anomalous increase in the number of *X* chromosomal elements both in male and female started and how it was reduced to basic number of *XO* in male and *XH* in female? (c) What could be the sex chromosome mechanism and genetic involvement of sex chromosome in sex determination? and so on.

Regarding the mechanism of increase of the number of *X* chromosomes in *L. grandis* BANERJEE (1959) without making any critical analysis opined that it was due to the fragmentation of the original single *X* in males only because he found no variation in oogonial number. In the present study the number was found to be variable in gonial cells of both sexes and during first spermatocyte division. The metrical data of chromosomes in plates with different *X* chromosomal numbers in metaphase I did not favour the origin of the higher *X* chromosomal numbers simply by successive fragmentation of the original single *X* as the different data were not corroborative. If some one was still inclined on the origin of the extra *X* elements by fragmentation, it could be argued that the anomaly in the data was due to sampling error, technical

shortcoming or even fragmentation was associated with other mechanism of structural and numerical changes. Anyhow the serious drawbacks of the fragmentational origin would be to explain the loss of genetic material contained in the original *X*, loss of fragments during anaphase I was expected to be seen as heteropteran chromosomes claimed to be with diffuse centromeric activity for experimentally induced fragments were viable according to different workers (see WHITE, 1973; UESHIMA, 1979; MANNA, 1983) but here it was behaving differently and so on. Therefore, we were not in favour of accepting the origin of the extra *X* chromosomal elements by fragmentation. It was very likely due to the process of chromatin elimination. Regular occurrence of different forms of chromatin elimination were on records in *Ascaris* (WALTON, 1924), *Chironomus* (KEYL & HAGELE, 1966), *Sciara* (DUBOIS, 1933), scale insects (BROWN, 1960; 1961) and even in a pyrrhocorid bug, *Iphita limbata* (MANNA & DEB-MALLICK, 1981b). In *I. limbata,* males had 9 *X* chromosomal elements and females had only 2. It was suggested by us (MANNA & DEB-MALLICK, 1981b) that the species had basically *XO*: *XX* sex chromosome mechanism but in males very likely before the first cleavage division after fertilization chromatin bodies were eliminated from the single original *X*. Thus nine *X* elements were traceable in all the spermatogonial plates and also upto metaphase II. Eight extra *X* elements showed sign of degeneration at second division of meiosis and the original *X* remained functional and was included in the *X*-bearing sperm while the other kind of sperm was free of the *X*. In *L. grandis* the *X* chromatin elimination was regular during development of both sexes instead of only in males of *I. limbata*. Anyhow the occurrence of anomalous number and size of the *X* elements in different gonial cells of both sexes of *L. grandis* has been suggested to be due to chromatin elimination rather than fragmentation because the process would not implicate the fundamental genetical problems associated with fragmentation like loss of genetic material and its regaining etc. focussed before. It would be legitimate to think about the significance of this uncommon phenomenon of *X* chromatin elimination leading to mosaic constitution of *X* chromatin elements in gonial cells of both sexes of *L. grandis*. Although there were different forms of sex chromatin elimination reviewed earlier (WHITE, 1973; MANNA & DEB-MALLICK, 1981b), the present type in *L. grandis* could be thought of the elimination of DNA of chromosomal origin as a gene amplification mechanism as suggested in other insects (STICH, 1962) including a Heteroptera species (MANNA & DEB-MALLICK 1981b). However, the necessity of the occurrence of these isolated instances was not resolved. As regards the time of origin of the extra *X* chromosomal elements in both sexes of *L. grandis* though it must have taken place during early cleavage division, precisely at what state of cleavage division was not known. Since the gonial numbers in both sexes were variable between 15 and 23 in males and 16 and 21 in females in different cells of the same individual, it could be suspected that the extra *X* chromosomal elements began to originate after a few divisions of the differentiation of the primordial germ cell. We were not sure if the variation of

the number of *X* chromosomal elements originated still earlier because we did not examine the chromosome number in tissues other than gonads of *L. grandis*. Since there were cells with different numbers due to extra *X* chromosomal elements ranging between 1 (original *X*) and 9 (1 original plus 8 *X*'s extra) in male and between 2 (original *X*'s) and 7 (2 original plus 5 *X*'s extra), the mosaic condition must have been reached by the divisional potentiality of the cells with different *X* chromosomal elements, if it did not originate by differential chromatin elimination process of the original *X*. Anyhow the differential rate of division of the gonial cells with different constitution of *X* elements could not be known as their intraindividual frequency was not determined for want of adequate number of dividing cells. The sex chromosome constitution in *L. grandis* has been advocated to be basically *XO* : *XX* and not any other type for more than one reason as in *Euryopthalmus humilis, E. rufipennis,* (PIZA, 1946, 1953), *Largus cinctus, L. succinctus* (WILSON, 1909) belonging to subfamily Larginae so far cytologically investigated has uniformly *XO* : *XX* sex chromosome mechanism, the variable number of *X* elements persisted upto metaphase I was invariably reduced to only one *X* and that underwent post-reduction at anaphase II in *L. grandis* like other heteropteran species. In species with X_1X_2O males in coreid bugs, in *Dysdercus* (Pyrrhocoridae) the two *X* chromosomes remained fused throughout spermatogenesis in the former and underwent fusion at anaphase I in *Dysdercus* (MANNA, 1951, 1956) but the reverse situation was seen in *L. grandis* and lastly the gonial complement had the minimlm of 15 chromosomes with one *X*, so also at metaphase I. Therefore, sex chromosome mechanism in *L. grandis* was fundamentally *XO* : *XX* on fertilization which underwent successive changes in number and size by a process of chromatin elimination which was rectified again to *XO* or else the chromatin elimlnation process of the *X* became inoperative in metaphase II in male and to *XX* in females possibly also at one of the oocyte divisions. The crucial proof of dissolution of all but the original *X* after first spermatocyte division in male was not available but this was not unexpected if the origin of the extra *X* chromosomal elements was by chromatin elininatian. However it remained unresolved whether the extra *X* chromosomal elements underwent regular division in gonial complements or else they were formed every time in most of the cell other than having original constitution of 15 in males and 16 in females. This could not be verified definitely because gonial anaphases were not studied while at anaphase I the extra elements underwent dissolution or else as suggested above the chromatin elimination of the *X* was inoperative. Therefore, further studies are in progress to verify several interesting points referred to before. Anyhow the behaviour of extra *X* chromosomal elements was unique and even more interesting than that of *I. limbata* (MANNA and DEB-MALLICK 1981b).

Acknowledgements: Grateful acknowledgement is made to the University Grants Commission, New Delhi for the financial assisstance.

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Department of Zoology, University of Kerala,
Kariavattom, Trivandrum 695 581

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Printed by V. K. K. Prabhu at GEO Printers, Plamood, Trivandrum 695 004
for the Association for Advancement of Entomology.